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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 394.01/A1

Topic: A.03. Postnatal Neurogenesis

Title: Dental stem cells of the apical papilla increase TRPA1 responses in trigeminal neurons

Authors: *M. ESKANDER, N. RUPAREL, A. DIOGENES
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Abstract: Regenerative endodontic procedures are biologically based procedures designed to restore normal physiologic function in the pulp-dentin complex by the delivery of mesenchymal stem cells (MSCs). Stem cells of the apical papilla (SCAP) are recognized as a prominent population of MSCs in these procedures. Many regenerative endodontic patients regain sensitivity to cold stimulation as early as one year post-procedure, even with a coronal restoration in place. However, the mechanisms mediating neuronal sprouting and transduction of physiologic stimuli (eg. cold sensation) after regenerative endodontic procedures is not fully understood. We have hypothesized that SCAP modulate nociceptive function through a paracrine mechanism that involves the release of soluble trophic factors and upregulation of TRPA1 function. To test this hypothesis, we established a co-culture system with a previously characterized human SCAP cell-line and rat primary trigeminal sensory neurons in a dual-chamber culture environment. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF) gene expression levels and release from SCAP co-cultured with TG neurons was measured by qRT-PCR and specific ELISA assays, respectively. In addition, the effect of SCAP co-culture on TRPA1 activity was evaluated using whole cell patch-clamp electrophysiology. SCAP co-cultured with TG neurons displayed significant upregulation of BDNF ($p<0.001$) and GDNF ($p<0.001$) mRNA. In addition, the release of BDNF was significantly higher than GDNF or NGF release ($p<0.001$). Finally, TG neurons co-cultured with SCAP demonstrated increased TRPA1 mediated (mustard oil-evoked) inward currents at 24 hours ($p<0.001$) and 48 ($p<0.05$) hours in co-culture compared to inward currents in neurons cultured alone. Collectively, these data suggest that SCAP responded to the nearby presence of neurons by the release of supportive trophic factors that in turn increased TRPA1-mediated responses. Importantly, this novel dental stem cell-neuronal interaction may underlie the mechanism of cold detection in the regenerated dental pulp.

Disclosures: **M. Eskander:** None. **N. Ruparel:** None. **A. Diogenes:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 394.02/A2

Topic: A.03. Postnatal Neurogenesis

Support: Italian Ministry of Health RF-2009-1543811

Catholic University D3.2 and D1 funds

Title: Involvement of pro-survival pathways and epigenetic mechanisms in extremely low-frequency electromagnetic field induced enhancement of adult hippocampal neurogenesis

Authors: *L. LEONE[1], S. FUSCO[1], M. V. PODDA[1], S. A. BARBATI[1], A. MASTRODONATO[1], D. D. LI PUMA[1], R. PIACENTINI[1], S. ZAFFINA[2], C. GRASSI[1]
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Abstract: In recent years, much effort has been focused to identifying strategies able to stimulate adult neurogenesis, a process that generates new neurons throughout life and that appears to be dysfunctional in the senescent brain and neurodegenerative diseases. We previously reported that *in vivo* exposure to extremely low-frequency electromagnetic fields (ELFEFs) promotes the proliferation and neuronal differentiation of hippocampal neural stem cells (NSCs) that functionally integrate in the dentate gyrus (DG). Here, we demonstrated that *in vivo* ELFEF stimulation also promotes the survival of neurons generated from NSCs residing in the hippocampal neurogenic niche. This effect is associated with down-regulation of the pro-apoptotic protein Bax and increased expression of the anti-apoptotic protein Bcl-2. As a result of increased survival of granule cells in the hippocampal DG, the ELFEF- exposed mice showed improved spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects on the process of adult neurogenesis, we extended our studies to an *in vitro* model of NSCs isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Collectively, our results further underscore the potential for the use of ELFEFs in

ameliorating the neurogenesis alterations associated with normal aging or neuropsychiatric and neurodegenerative diseases. Moreover, our study identifies a novel molecular mechanism responsible for ELFEF-induced enhancement of hippocampal neurogenesis, that could be targeted in future stem cell-based therapeutic approaches.

Disclosures: **L. Leone:** None. **S. Fusco:** None. **M.V. Podda:** None. **S.A. Barbati:** None. **A. Mastrodonato:** None. **D.D. Li Puma:** None. **R. Piacentini:** None. **S. Zaffina:** None. **C. Grassi:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: Fondecyt-11110012

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Fondecyt-1120156

Title: Wnt receptors Frizzled in neural progenitor cells and immature neurons of the adult hippocampus

Authors: ***L. VARELA-NALLAR**[1], G. A. ANDAUR[1], M. D. MARDONES[1], M. VARAS-GODOY[2], N. C. INESTROSA[3]
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Abstract: Wnts are secreted glycoproteins that have distinct roles during early development, and also they are key regulators of late embryonic and postnatal development of the central nervous system. In the adult brain, the Wnt signaling pathway regulates neurogenesis in the subgranular zone (SGZ) of the hippocampal dentate gyrus. In this region, Wnt ligands have shown to regulate both, proliferation and differentiation of neural progenitor cells. Here, we investigated the role of Wnt receptors Frizzled (FZD) in adult neurogenesis. In mammals, 10 FZD receptors have been identified and these may activate different signaling cascades in response to a specific Wnt

ligand. The presence of FZD receptors in the SGZ was studied by immunofluorescence staining in brain sections of 2-month-old wild-type mice and also in cultured adult hippocampal progenitors (AHPs) isolated from adult mouse brain. In the hippocampus, neural progenitor cells and newborn immature neurons were identified by immunodetection of protein markers. We determined that FZD3 is present in neural progenitor cells in the SGZ, and it was also observed in cultured AHPs. On the other hand, FZD1 was observed in progenitor cells but was mainly detected in immature neurons in the dentate gyrus, suggesting that the expression of this receptor is increased during differentiation. In AHPs, overexpression of FZD1 induced a decrease in the levels of the progenitor cell markers Nestin and SOX2, suggesting that differentiation was induced. Finally, retroviral shRNA-mediated knock-down of FZD1 in the dentate gyrus decreased neuronal differentiation of progenitor cells. Our findings suggest that there is a temporal expression of FZD receptors during adult hippocampal neurogenesis, and these receptors could mediate specific Wnt effects during the development of newborn neurons. Also, our results indicate that FZD1 regulates the differentiation of neural progenitors cells in the adult hippocampus.

Disclosures: L. Varela-Nallar: None. **M. Varas-Godoy:** None. **N.C. Inestrosa:** None. **G.A. Andaur:** None. **M.D. Mardones:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 394.04/A4

Topic: A.03. Postnatal Neurogenesis

Support: Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009521)

Title: Neuroprotection with antioxidant anthocyanins against ethanol-induced oxidative stress and neurodegeneration via PI3K/Akt/GSK3β pathway

Authors: *G. YOON, S. SHAH, M. KIM
Life Sci., Gyeongsang Natl. Univ., Jinju, Korea, Republic of

Abstract: Oxidative stress has been implicated in the pathophysiology of several neurodegenerative disorders. Numerous studies have reported that ethanol exposure produces reactive oxygen species (ROS), one of the most noted molecular mechanisms of ethanol

neurotoxicity. We recently reported $GABA_{B1}$ receptor dependent protection of anthocyanins against ethanol-induced apoptosis in prenatal hippocampal neurons. Here, we sought to extend these findings by examining the effect of anthocyanins neuroprotection against ethanol in the hippocampus of postnatal day 7 rat brain. After four hours of ethanol alone and along with anthocyanins administration, the expression of glutamate receptors (AMPARs), intracellular signaling molecules, different synaptic, inflammatory and apoptotic markers were evaluated. The results indicate that anthocyanins significantly reversed ethanol-induced inhibition of glutamatergic neurotransmissions, synaptic dysfunction, $GABA_{B1}R$ activation and neuronal apoptosis by stimulating PI3K/Akt/GSK3β pathway in the hippocampus of postnatal age brain. Anthocyanins also inhibited ethanol activated phosphorylated c-Jun N terminal kinase p-JNK, phospho Nuclear Factor Kappa B (p-NF-kB), Cyclooxygenase 2 (COX 2) expressions, attenuated neurodegeneration and neuronal cell death in the hippocampal CA1 region of developing brain. Furthermore anthocyanins increased cell viability, attenuated ethanol-induced PI3K dependent ROS production, cytotoxicity and caspase-3/7 activation *in vitro*. In summary these results indicates that anthocyanins are beneficial against ethanol abuse in the developing stage.

Disclosures: G. Yoon: None. **S. Shah:** None. **M. Kim:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: NSERC Discovery Grant 371716-2009

Ministry of Research and Innovation Early Researchers Award ER10-07-122

Ontario Graduate Scholarship

Title: The requirement of Bcl-2 in neuronal stem and progenitor cell development in the adult brain

Authors: *M. CEIZAR, J. DHALIWAL, M. SMALLWOOD, Y. XI, D. LAGACE
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Abstract: A large proportion of neuronal stem and dividing progenitor cells (NSPCs) in the adult brain die due to apoptotic cell death. Elucidating at what stage during NSPCs development apoptosis occurs and the mechanisms that regulate apoptosis would aid in promoting the regenerative therapeutic potential of adult neurogenesis. Knockout mouse models suggest that the B-cell Lymphoma-2 (Bcl-2) family of pro-apoptotic (e.g. Bcl-2-associated X (BAX)) and anti-apoptotic (e.g. Bcl-2) proteins are regulators of NSPC death through activation of the intrinsic mitochondrial apoptotic pathway. To evaluate the role of Bcl-2 in NSPCs throughout their development, Bcl-2 was removed from NSPCs within the adult brain *in vivo*. Retroviral mediated gene transfer of Cre directly into the dentate gyrus of floxed Bcl-2 (fBcl-2) mice resulted in the complete ablation of neurogenesis by 30 days post infection supporting that Bcl-2 is essential for survival of the dividing progenitor cells. This was also confirmed through removal of Bcl-2 using a nestin-induced CreER^{T2} conditional mouse. Conditional ablation of Bcl-2 reduced the total number of recombined PCs and increased the number of activated caspase-3 (AC3) expressing cells by 12 days following induction of recombination. Additionally removal of Bcl-2 reduced the GFAP+/SOX2+ stem cell population in hippocampus, suggesting that Bcl-2 is necessary for stem cell maintenance. In agreement with the role of Bcl-2 acting upstream of BAX, the combined removal of Bcl-2 and BAX was able to rescue the Bcl-2 mediated ablation of neurogenesis. Together, these finding support the requirement of Bcl-2 and BAX in stem cells and the generation of newborn neurons in the adult brain.

Disclosures: M. Ceizar: None. **J. Dhaliwal:** None. **M. Smallwood:** None. **Y. Xi:** None. **D. Lagace:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: AXA fellowship (CC)

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Title: Differential oxygen tension in the SGZ niche determines the early survival of newborn hippocampal granule cells

Authors: *C. CHATZI[1], E. SCHNELL[2,3], G. WESTBROOK[1]
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Abstract: The subgranular zone (SGZ) of the adult hippocampal dentate gyrus (DG) is one of the two neurogenic niches in the mammalian brain where new neurons are continuously generated from neural stem cells and progenitors. In the adult mouse as many as 4000 new granule cells are born daily, but only a small subset of those (~30%) survive the 4 weeks post-mitosis to differentiate into mature granule cells. To survive, adult-generated granule cells must pass through two critical periods: an early one during transition from intermediate progenitors to neuroblasts, and a later during integration of immature neurons into pre-existing hippocampal circuits. Although the integration phase is activity-dependent, little is known about the mechanisms responsible for early survival during the first week post-mitosis. Given the increasing evidence that differential oxygen tensions may be an important component of the neurogenic niche, and that oxygen-sensing may regulate the survival of neural stem cell precursors, we tested the role of oxygen tension on early granule cell survival. In order to characterize the oxygen tensions within the SGZ, we injected adult mice with the hypoxia marker pimonidazole hydrochloride. Several hypoxic niches were detected along the inner border of the SGZ with the hilus, consisting of adult neural stem cells and early intermediate progenitors. Interestingly, oxidative byproducts, marked by 8-hydroxyl-2′-deoxyguanosine, were highly localized in late intermediate progenitors and neuroblasts located adjacent to the hypoxic areas. Thus, we hypothesized that migration of a subset of intermediate progenitors along differential oxygen gradients might result in oxidative damage and thus trigger an early phase of apoptosis. In order to test this idea, we employed a hypoxia mimetic agent, DiMethylOxallyl Glycine (DMOG), that stabilizes Hypoxia Inducible Factor-1α (HIF-1α), the major regulator of oxygen homeostasis under normoxia. Mimicking hypoxia with DMOG specifically promoted the survival of the DG newborn cells during their transition from intermediate progenitors to neuroblasts (PND 3-7). However, DMOG had no effect on proliferation and differentiation both *in vivo* and *in vitro*. Using whole-cell recording of 3 day-old DG progenitors and immunohistochemical markers of neural activity, there was no detectable neural activity at this early stage, suggesting that the action of DMOG on survival was activity-independent. We propose that differential oxygen tensions within the SGZ contribute to the neurogenic microenvironment and determine the early survival of adult DG newborn cells.

Disclosures: **C. Chatzi:** None. **E. Schnell:** None. **G. Westbrook:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: NINDS award 040726B1 to MMM

Title: MicroRNA expression in the early postnatal hippocampus of the rat differs between the sexes and is regulated by estradiol and DNA methylation

Authors: *K. E. KIGHT[1], J. M. BOWERS[2], M. M. MCCARTHY[2,3]
[1]Program in Mol. Med., [2]Pharmacol., [3]Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neurogenesis is regulated by numerous extrinsic and intrinsic factors, and varies among brain regions and across the lifespan. We have previously reported that early postnatal neurogenesis in the hippocampal formation of the Sprague Dawley rat differs between males and females, and is regulated by steroid hormones. Cell proliferation is greater in the dentate gyrus of males compared to females during the first week of life, but not the second, and this is modulated by estradiol signaling (Bowers et al., 2010). The downstream factors mediating the sex difference in cell genesis and the cell cycle response to estradiol in the early postnatal hippocampus are as yet unknown. In this study we examined the expression of several microRNAs known to be functionally involved in neuronal cell genesis, differentiation, and maturation. Female rat pups had higher baseline expression of several mature microRNAs in the dentate gyrus during the first postnatal week, including miR124, which is known to be involved in cell-cycle exit and neuronal differentiation. Exogenous administration of estradiol decreased expression of several microRNAs in the female dentate, while blocking estradiol signaling in males with tamoxifen increased microRNA expression. The effects of estradiol and tamoxifen on microRNA levels are inversely correlated with their effects on cell proliferation. In females, the increased cell proliferation in the early postnatal dentate in response to estradiol was blocked by inhibition of DNA methylation using zebularine, and zebularine also increased expression of miR124 and other microRNAs in the female dentate. Blocking DNA methylation with zebularine had little effect on expression of these microRNAs in the dentate of male pups. These results demonstrate a sex difference in microRNA expression during early postnatal development of the hippocampus, corresponding to a period in which a robust sex difference in cell genesis is seen. These results also suggest that microRNAs may be involved in mediating the effects of hormonal

signaling and epigenetic factors on cell proliferation and neurogenesis in the developing hippocampus. This work is supported by NINDS award 040726B1 to MMM.

Disclosures: K.E. Kight: None. **J.M. Bowers:** None. **M.M. McCarthy:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: TL1R000108

TL15TL1RR025739

Title: BMP signaling regulates the tempo of adult hippocampal progenitor maturation at multiple stages of the lineage

Authors: *A. M. BOND[1], C.-Y. PENG[1], E. A. MEYERS[1], T. MCGUIRE[1], O. EWALEIFOH[2], J. A. KESSLER[1]
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Abstract: Novel environmental stimuli, such as running and learning, increase proliferation of adult hippocampal neural stem cells (NSCs) and enlarge the population of new neurons. However, it remains unclear how increased numbers of new neurons can be generated in a time frame far shorter than the time required for proliferating stem cells to generate these neurons. Here we investigated how bone morphogenetic protein (BMP) signaling regulates neural progenitor cell maturation in the adult hippocampus. BMPs are a class of morphogens within the transforming growth factor β superfamily, and are known to negatively regulate hippocampal neurogenesis. We show that BMP signaling in the SGZ regulates the tempo of neural progenitor cell (NPC) maturation by directing their transition between states of quiescence and activation at multiple stages along the lineage. Virally mediated overexpression of BMP4 caused NPC cell cycle exit and slowed the normal maturation of NPCs, resulting in a long-term reduction in neurogenesis. Conversely, overexpression of the BMP inhibitor noggin promoted NPC cell cycle entry and accelerated NPC maturation. Similarly, BMP receptor type 2 (BMPRII) ablation in Ascl1+ intermediate NPCs accelerated their maturation into neurons. This in turn resulted in a

period of increased neurogenesis that is associated with enhance cognition on a hippocampus dependent task. Thus inhibition of BMP signaling is a mechanism for rapidly expanding the pool of new neurons in the adult hippocampus by tipping the balance between quiescence/activation of NPCs and accelerating the rate at which they mature into neurons.

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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: NWO VIDI grant H64.09.016 to CPF

Title: Mitochondrial pro-apoptotic proteins are target of cooperative microRNA regulation in early stages of adult hippocampal neurogenesis induction after status epilepticus

Authors: *M. SCHOUTEN[1], S. A. FRATANTONI[2], C. J. Y. HUBENS[3], S. R. PIERSMA[2], T. V. PHAM[2], E. VREUGDENHIL[3], P. J. LUCASSEN[1], C. P. FITZSIMONS[1]
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Abstract: The total outcome of adult hippocampal neurogenesis (AHN) is tightly regulated both by cell proliferation and apoptosis. IP administration of KA at doses that induce status epilepticus (SE) induce AHN starting at three days after treatment remaining for as long as 15 days in mice (1). We hypothesize that during the early stages of AHN induction after KA-induced status epilepticus (SE) a dysbalance in apoptosis-related proteins stabilizes mitochondrial integrity, subsequently rescuing newborn neurons from apoptosis. By means of proteomics, transcriptomics and microRNAomics analyses we identified several apoptosis related targets and microRNAs that were differentially expressed in the dentate gyrus 3 days after KA treatment. Interestingly, while protein levels of some members of the BCL family of apoptosis-related proteins were decreased, mRNA levels remained unchanged, suggesting a possible role for microRNA regulation. This role was confirmed in hippocampal neural stem cell (NSC) cultures. Our data shows that specifically upregulated microRNAs bind to the 3'UTR of mRNA of members of the BCL family and inhibit protein expression. Furthermore,

overexpression of specific microRNAs and concomitant downregulation of their targets revealed a significant contribution to lowering KA-associated caspase3 activity in NSCs. As observed before, low amounts of caspase3 activity induced a differentiation phenotype (2). These data support our hypothesis that NSC apoptosis is hampered shortly after induction of SE and suggests microRNA upregulation as an intrinsic regulatory mechanism to favor differentiation over induction of apoptosis. This work was financed by a NWO VIDI grant H64.09.016 to CPF. (1) Jessberger et al., Exp Neurol, 2005 (2) Fitzsimons et al., Mol Psych, 2013

Disclosures: **M. Schouten:** None. **S.A. Fratantoni:** None. **C.J.Y. Hubens:** None. **S.R. Piersma:** None. **T.V. Pham:** None. **E. Vreugdenhil:** None. **P.J. Lucassen:** None. **C.P. Fitzsimons:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: The Research Funding for Longevity Sciences (23-1, 26-25) from NCGG, Japan

Title: Chk2 maintains neural stem cell pool in mouse adult hippocampus during aging

Authors: K. IBARAKI[1], M. SAWADA[2], K. SAWAMOTO[2], M. MINAMIYAMA[1], W. MARUYAMA[1], *N. MOTOYAMA[1]
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Abstract: Adult neurogenesis occurs throughout life in the subgranular zone (SGZ) of the dentate gyrus of hippocampus and the subventricular zone (SVZ) lining the lateral ventricles in the adult mammalian brain. Newborn neurons in SGZ integrate into the neural circuit, thereby implicating in learning and memory. The adult neurogenesis is declined with age, however, it still remains unknown about the mechanisms of age-related decline in neurogenesis. Checkpoint kinase 2 (Chk2), a mediator of DNA damage response, is essential for p53-mediated apoptosis and cell cycle arrest in response to DNA damage. Here we show that Chk2 deficiency accelerates a decrease in the neural stem cells in SGZ, leading to a decline in hippocampal neurogenesis. The weight of the body and brain of 4.5-month-old Chk2 KO mice was indistinguishable from those of wild type mice. Quantitative analysis in SGZ reveals that the number of S100beta-,

Sox2+ neural stem cells and DCX+ immature newborn neurons is significantly reduced in the 4.5-month-old Chk2 KO mice compared to that of wild type mice. The ratio of differentiation from neural stem cells to immature neurons in Chk2 KO mice is comparable to that of wild type mice. These results suggest that Chk2 may be required for the maintenance for adult hippocampal neural stem cell pool and cognition during aging. Supported by The Research Funding for Longevity Sciences (23-1, 26-25) from NCGG, Japan

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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 394.11/A11

Topic: A.03. Postnatal Neurogenesis

Title: Neuroblasts without a neurogenic niche maintain adult neurogenesis on the second stage of the central olfactory pathway in the shore crab, Carcinus maenas

Authors: *M. SCHMIDT, C. D. DERBY
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Abstract: Adult neurogenesis persists in the central olfactory pathway of the decapod crustacean brain. The first stage in this pathway is the olfactory deutocerebrum (OD), which receives afferent input from olfactory receptor neurons. Adult neurogenesis in the OD is maintained by a few putative neural stem cells, identified as 'adult neuroblasts' (aNBs). Each aNB is associated with a small group of neuronal progenitor cells located in a proliferation zone (PZ) within the paired neuronal soma clusters of the OD: the lateral cluster (LC) containing the somata of ascending projection neurons (PNs) and the medial cluster containing the somata of local interneurons (LNs). In spiny lobsters Panulirus argus and crayfish Procambarus clarkii, each aNB of the OD is embedded in a distinct aggregate of small, bipolar cells likely representing a neurogenic niche (Schmidt, J Comp Neurol 503:64-84, 2007; Song et al., Arthrop Struct Dev 38:339-360, 2009; Schmidt & Derby, J Comp Neurol 519:2283-2319, 2011). The second stage of the central olfactory pathway is the lateral protocerebrum (LP), which is the target of ascending OD-PNs. The LP contains the hemiellipsoid body (HB), a neuropil corresponding to the mushroom body of insect brains (Wolff et al., J Comp Neurol 520:2824-2846, 2012). The somata of intrinsic HB-LNs form a large soma cluster attached to the HB, the HB cluster (HBC).

In anomuran and brachyuran crabs including Carcinus maenas, the HBC contains a circumscribed PZ (Schmidt, Brain Res 762:131-143, 1997). We examined if the PZs in the LC and HBC of the brain of adult C. maenas are associated with aNBs embedded in neurogenic niches by combining *in vivo* labeling using the S-phase marker BrdU with nuclear labeling. Three BrdU injections given in 8 h-intervals with a total survival time of 24 h resulted in the labeling of (1) neuronal progenitor cells in the PZs of LC and HBC, (2) cells associated with a neurogenic niche in the LC, and (3) one large cell at the edge of the PZ in the HBC (n = 6 crabs). Significantly, this cell, which we identify as a putative aNB based on being larger than the cells in the PZ of the HBC, was not associated with a cellular aggregate reminiscent of a neurogenic niche. We conclude that adult neurogenesis in the LC and HBC of shore crabs is based on aNBs, which in the LC are associated with a neurogenic niche whereas in the HBC they are not. The latter result tightly links adult neurogenesis in the HBC of shore crabs to adult neurogenesis in the mushroom bodies of insect brains, where Kenyon cells, the intrinsic mushroom body LNs, are generated by the activity of large aNBs not associated with a neurogenic niche (Dufour & Gadenne, J Comp Neurol 495:635-643, 2006)

Disclosures: M. Schmidt: None. **C.D. Derby:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: 1R01NS067289-01A1

Title: Lncrnas in granule cell progenitor differentiation

Authors: *C. PENAS[1], V. STATIAS[3], J. CLARKE[4], M. ZHANG[5], N. AYAD[2]
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Abstract: We have performed a systems level analysis of cell cycle exit in the developing nervous system. We chose the granule cell progenitor (GCP) as a model to study cell cycle exit due to its abundance in the mammalian brain and importance to cerebellar development. We

performed RNA sequencing of GCPs at various times of differentiation and identified several long noncoding RNAs (lncRNAs), which are differentially expressed as GCPs exit the cell cycle. Considering the complexity of the "dark matter" RNA (produced by the 70% of the genome that does not encode proteins) and the relative high mass of that RNA in cells compared to the protein-coding RNA, it is logical to assume that lncRNAs harbor yet uncharacterized regulators of basic cellular processes, such as the cell cycle. Yet no systematic analysis of the contribution of long coding RNAs to cell cycle exit has been performed. We have performed functional studies, which demonstrate the contribution of long noncoding RNA to cell cycle exit of GCPs *in vitro*, *ex vivo*, and *in vivo*. Further, we have utilized long noncoding RNA- RNA interactions to generate a statistical model of cell cycle exit in the developing nervous system.

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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Alzheimer's Society Research Program Doctoral Award

Dr. and Mrs. Albert Spatz Doctoral Award

Title: The presenilins are not required for cell intrinsic regulation of adult hippocampal neurogenesis

Authors: *M. VACULIK[1], J. DHALIWAL[1], K. L. KUMAR[1], A. MAIONE[1], T. KANNANGARA[1], J.-C. BÉÏQUE[1], J. SHEN[2,3], D. C. LAGACE[1]
[1]Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [2]Neurol., Brigham and Women's Hosp., Boston, MA; [3]Neurosience, Harvard Med. Sch., Boston, MA

Abstract: Presenilins (PS1 and PS2) are components of the gamma-secretase complex and mutations in these genes are the major cause of familial Alzheimer's disease (AD). Cognitive decline in AD and the role of PSs in embryonic neurogenesis raised the possibility that reduced neurogenesis may contribute to AD-associated cognitive deficits. This was further supported by

conditional deletion of PSs in the adult cortex resulting in learning and memory impairments, synaptic deficits and age-dependent neurodegeneration. These findings prompted us to investigate whether PSs alter hippocampal neurogenesis in the adult brain. To birthmark and track the fate of dividing progenitor cells (PCs) in the dentate SGZ, we injected a mixture of retroviral GFP-Cre (*CAG-GFP-Cre)* and control RFP (*CAG-RFP)* into floxed *PS1* mice in the *PS2*-null background. The resulting *PS*-null GFP+ PCs developed normally and within 1 month the majority of PCs had a mature granule cell phenotype, measured by expression of NeuN, dendritic morphology and electrophysiological assessment including the AMPAR/NMDAR ratio. Exercise also induced an increase in the *PS*-null GFP+ and control RFP+ cells in animals that had 2 weeks of access to a running wheel compared to animals housed with a locked wheel, suggesting *PS* does not alter exercise induced increase in adult neurogenesis. To evaluate further whether *PSs* are required for adult neurogenesis, we generated a *PS* conditional KO (cKO) mouse carrying *NestinCre-ER*^{T2}, *RosaYFP*, homozygous floxed *PS1*, and *PS2*-null alleles. At five weeks of age, *PS* cKO mice and littermate controls received tamoxifen to induce recombination in the *Nestin* expressing stem/progenitor cells and their progeny. Compared to controls, the number of recombined (YFP+) cells co-expressing the stem cells markers (Sox2 and GFAP) or Ki67-expressing proliferating cells was unchanged in *PS* cKO mice. Additionally, up to 60 days following removal of PSs, the number of mature neurons expressing NeuN was also normal. Together these results indicate that loss of PSs does not affect proliferation, survival, and maturation of neurons generated within the adult hippocampus, suggesting a divergent role for PSs in the embryonic versus adult neurogenesis.

Disclosures: **M. Vaculik:** None. **J. Dhaliwal:** None. **K.L. Kumar:** None. **A. Maione:** None. **T. Kannangara:** None. **J. Béïque:** None. **J. Shen:** None. **D.C. Lagace:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 394.14/A14

Topic: A.03. Postnatal Neurogenesis

Title: Regulation of the process of adult neurogenesis in the adult hippocampal dentate gyrus by Parvalbumin-positive GABAergic neurons

Authors: *H. MIWA[1,2], N. TAMAMAKI[3], Y. YANAGAWA[1,2]
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Technol. Agency, CREST, Tokyo, Japan; [3]Dept Morphological Neural Science, Kumamoto Univ., Kumamoto, Japan

Abstract: Adult neurogenesis occurs throughout life in discrete regions of the mammalian brain. In the hippocampal dentate gyrus (DG), immature neurons, originating from adult neural progenitors at the subgranular zone, migrate into the granule cell layer to become new dentate gyrus granule cells. Adult neurogenesis is highly regulated by physiological and pathological stimuli, including exercise, environmental enrichment and seizures. One of the well-established mechanisms for regulation of the process of adult neurogenesis is activity to newborn neurons using the neurotransmitter γ-aminobutyric acid (GABA). Several recent studies have suggested that GABA has crucial roles in regulating different steps of adult neurogenesis, including proliferation of neural progenitors, migration and differentiation of neuroblasts, and synaptic integration of newborn neurons. GABAergic neurons in the DG are a diverse group that can be classified by a variety of morphological, neurochemical, and physiological characteristics. Parvalbumin (PV)-containing GABAergic neurons project axon terminals that synapse exclusively on the axon initial segments to control the output, synchronizing the action potential firing of large group of principal neurons. Furthermore, PV-positive GABAergic neuron activity regulates Type I stem cell quiescence via tonic GABA receptor signaling. To investigate the roles of PV-positive GABAergic neurons in postnatal neurogenesis, we generated PV-positive neuron-specific GAD67 knockout mice using the Cre-loxP system. PV-Cre; GAD67flox/flox mice showed reductions in expression of calbindin, a mature granule cell marker, but similar levels in expression of calretinin, a immature granule cell marker, compared to the control GAD67flox/flox mice. These data suggest that PV-positive GABAergic neurons have a critical role in the maturation of newborn neurons during postnatal dentate gyrus neurogenesis.

Disclosures: H. Miwa: None. **N. Tamamaki:** None. **Y. Yanagawa:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

Support: AA020023

AA020024

AA019767

AA007573

Title: Histone deacetylase 1 is required for doublecortin expression: implication for ethanol inhibition of neurogenesis

Authors: *J. Y. ZOU[1], F. T. CREWS[2]
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Abstract: Histone deacetylases (HDACs) are nuclear enzymes capable of repressing gene expression by removing acetyl groups from histones and play important role in maintaining functional neurogenesis, but little is known about the functional specificity of different HDAC isoforms in regulation of ethanol-impaired hippocampal neurogenesis. The present study investigated the specific role of HDAC isoforms in regulation of hippocampal neurogenesis under control as well as during ethanol exposure in an organotypic hippocampal-entorhinal cortex (HEC) slice culture model. HEC slices were prepared from P7 neonates, maintained in culture for two weeks and then treated with pan-HDAC inhibitors including trichostatin A (TSA), sodium butyrate (SB) and valproic acid (VPA) and specific HDAC1 inhibitor MS-275. DCX immunoreactivity (IR) and BrdU+ neural progenitors were measured with immunohistochemistry. Treatments of HEC slices with pan-HDAC inhibitors SB (100uM) and VPA (100uM) for 4 days significantly increase DCX+IR and proliferation of BrdU+ progenitors. Treatment of HEC slices with specific HDAC1 inhibitor MS-275 (500nM) drastically inhibited DCX expression and progenitor cell proliferation. HDAC1 knockdown with specific siRNA had the same effects on DCX expression as MS-275. Combination of MS-275 with pan-HDAC inhibitors SB and VPA eliminates the increased effects of SB and VPA on DCX expression. However, combined treatments of MS-275 and HDAC1 activator such as acetyl-CoA reversed MS-275-induced reduction of DCX expression. These results strongly suggest that HDAC1 is required for maintaining hippocampal neurogenesis. Ethanol (100mM) treatment for 4 days significantly reduced DCX expression and proliferation of neural progenitors accompanying with reduction of HDAC1 mRNA expression. The presence of MS-275 during ethanol treatment further decreased DCX expression. Ethanol inhibition of DCX expression was also reversed by HDAC1 activator Acetyl-CoA. Western blot analysis further indicates that ethanol exposure results in mobilization of HDAC1 from nuclear to cytoplasmic compartments, suggesting reduced nuclear HDAC1 activity. Together, these novel findings suggest that HDAC1 is required for hippocampal neurogenesis and may be a therapeutic option for ethanol-impaired neurogenesis.(Supported by NIAAA)

Disclosures: J.Y. Zou: None. **F.T. Crews:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Title: Transcription factors Coup-TFI and Coup-TFII regulate the migration and survival of Pax6-expressing granule cells of the olfactory bulb

Authors: *Z. XING, J. LI, Z. XU, Q. LIANG, Z. LIU, Z. YANG
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Abstract: In the adult brain, the main function of SVZ neural stem cells is generating interneurons for the olfactory bulb (OB) throughout life. In general, the slowly dividing B1 cells give rise to rapidly dividing transit amplifying C cells, which in turn generate large numbers of immature neurons (also known as neuroblasts, or A cells) that migrate forwards along the rostral migratory stream (RMS) to the OB, where they become olfactory mature interneurons. In the present study, we have examined the function of chicken ovalbumin upstream promoter transcription factors COUP-TFI and COUP-TFII in regulation of mouse OB interneuron migration and survival. We found that COUP-TFI, but not COUP-TFII, is expressed in the SVZ stem/progenitor cells, migrating neuroblasts and mature OB interneurons. Conditional inactivation of COUP-TFI resulted in upregulation of COUP-TFII expression in the SVZ-RMS-OB pathway. In COUP-TFI/COUP-TFII double conditional mutants, we found that Pax6-expressing mature interneurons in the granular cell layer were lost. We also found that NeuN-expressing cells accumulated in the double mutant SVZ. Accordingly, apoptotic cells were significantly increased in COUP-TFI/COUP-TFII double conditional mutant SVZ. These results suggest that Coup-TFI and Coup-TFII regulate the migration and survival of a subpopulation of OB granule cells. **Keywords**: Sp8; Dlx5/6; neural stem cells; neurogenesis; parvalbumin; interneurons; olfactory bulb

Disclosures: Z. Xing: None. **J. Li:** None. **Z. Xu:** None. **Q. Liang:** None. **Z. Liu:** None. **Z. Yang:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Title: Serotonin 1a receptor in sex-specific neonatal hippocampal development and later-life mood disorders: Possible cooperation with gpr30

Authors: *P. BANERJEE[1], S. SAMADDAR[2], A. MARSILLO[2], R. SCHRODER[1,2]
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[2]Doctoral Program in Biol., CUNY Grad. Ctr., New York, NY

Abstract: The neurotransmitter serotonin (5-HT) plays an important role in mood disorders and its synthetic rate is lower in the brain of women than men. Intriguingly, mood disorders are twice as common in women than men. It has been demonstrated that 5-HT signaling through 5-HT1A receptors (5-HT1A-R) is crucial for early postnatal hippocampal development and later-life behavior. Although 5-HT signaling through 5-HT1A receptors (5-HT1A-R) regulates early brain development, the mechanistic underpinnings have remained unclear. We have shown that suprabasal 5-HT1A-R signaling in postnatal day 6 (P6) mice through protein kinase Cε (PKCε) and extracellular receptor activated kinase 1/2 in the dentate gyrus (DG) boosts neonatal neuroblast proliferation in both sexes. However, the basal 5-HT1A-R signaling exerts a female-specific effect and neuroproliferation is severely impaired in P6 female but not male 5-HT1A-R(-/-) (KO) mice. Establishing the sex-specific developmental importance of early neuroproliferation, the KO female but not male mice showed significantly elevated anxiety-like behavior at P60. Intrahippocampal infusion of the selective PKCε stimulator DCP-LA at P6 partially rescued neuroblast proliferation in the KO females and repeated systemic treatment from P6 to P14 corrected the later-life anxiety-like behavior. To explore the possible mechanism of this sex-specific effect of 5-HT1A-R signaling, we studied the involvement of the estradiol receptor GPR30, which has been reported to associate with the 5-HT1A-R and also influence its signaling activity. 5-HT1A-R-mediated neuroproliferation in the P6 hippocampus was eliminated in the presence of the GPR30 antagonist G15 and the GPR30 agonist G1 elicited a dramatic increase in neuroproliferation. GPR30 has been believed to localize in the intracellular membranes. Our studies will investigate the possibility of its interaction with the 5-HT1A-R through both direct association as well as interaction with the signal transducers, such as G-proteins and effector molecules.

Disclosures: **P. Banerjee:** None. **S. Samaddar:** None. **A. Marsillo:** None. **R. Schroder:** None.

Poster

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Support: NIH Grant R01NS020013

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Title: Cross-talk between beta1-integrin and bone morphogenetic (BMP) signaling in the regulation of adult neurogenesis

Authors: *S. M. BROOKER, H. A. NORTH, C.-Y. PENG, T. L. MCGUIRE, J. A. KESSLER
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Abstract: The continuous generation of new neurons occurs throughout life in two localized regions of the adult mammalian brain, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Behavioral studies in rodents have suggested that ongoing hippocampal neurogenesis may play a key role in cognition. Diverse signaling molecules present in the SGZ regulate multiple aspects of the neurogenic process, including neural stem cell (NSC) maintenance, proliferation, and differentiation. In particular, inhibition of BMP signaling promotes hippocampal neurogenesis and improves cognition. However, relatively little is known about how different signaling pathways may interact to regulate NSC behavior. We found that BMP signaling in NSCs is modified by β1-integrin, an extracellular matrix-interacting protein that has been implicated in maintenance of NSCs. Co-immunoprecipitation studies demonstrated that β1-integrin physically interacts with both type I BMP receptors (BMPR1a and BMPR1b) in both the wild type adult hippocampus and in cultured NSCs. We used the Cre/LoxP system to genetically ablate β1-integrin in cultured NSCs derived from the SVZ of β1-integrin floxed mice. Ablation of β1-integrin in NSCs resulted in increased partitioning of BMPR1b into lipid rafts, increased pSMAD1/5/8 and p38 signaling, and increased expression of BMP target genes including GFAP and inhibitor of differentiation (ID) proteins. Disruption of lipid rafts resulted in a marked reduction in BMP signaling, indicating that lipid rafts are critical for BMP signaling in NSCs. We conclude that β1-integrin modulates BMP signaling in neural stem cells *in vitro* by regulating the localization of BMP receptors within the cell membrane.

Disclosures: **S.M. Brooker:** None. **H.A. North:** None. **C. Peng:** None. **T.L. McGuire:** None. **J.A. Kessler:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: NRF-2013R1A1A3010216

Title: Differential expression of hyperpolarization-activated and cyclic nucleotide-gated (HCN) channel isoforms during hippocampal development in the mouse

Authors: ***H. SEO**[1,2], M.-J. SEOL[1], K. LEE[1]
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Abstract: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are intermembrane proteins that serve as nonselective ligand-gated cation channels in the plasma membranes of heart and brain cells. Among HCN channels isoforms 1, 2, 3, and 4, HCN1, 2, and 4 are widely expressed throughout the brain and help to control the rhythmic activation of pacemaker oscillatory neurons during brain development as well as to control neural synaptic plasticity in the adult brain. In the mouse, the hippocampus has been studied extensively as a part of brain responsible for learning and spatial memory related to synaptic plasticity. Therefore, we examined the expression profile of brain-enriched HCN1, HCN2, and HCN4 in the hippocampus of mouse by ontogenic study with the tissue obtained from postnatal day (PND) 0, 7, 21, and 56. All of HCN1, HCN2, and HCN4 are expressed abundantly through the whole hippocampal area at PND 0 and PND 7 and each HCN channel showed the specific expression according to the hippocampal layer after PND 7. Especially, HCN4 expressions are collocalized with GFAP-positive immunoreactivity in stratum lacunosum moleculare layer at only PND 7. In PND 21 and PND 56, all of HCN1, 2 and 4 were outstandingly expressed at the pyramidal cells of all CA fields and parvalbumin-positive interneuron primarily at CA3. In the dentate gyrus, the immunoreactivity for HCN1, 2, and 4 are exhibited at hilus and granular cells near molecular layer, but not at double-cortin (DCX)-positive cells of subgranular zone in both PND 21 and PND 56. Taken together, the mapping process we performed to discovery the hippoocampal

expression pattern of HCN isoforms in mouse suggest the specific expression and distinct potential role of hippocampal HCN1, HCN2, and HCN4 during neural development.

Disclosures: **H. Seo:** A. Employment/Salary (full or part-time):; Kyungbook National University of medicine. **M. Seol:** None. **K. Lee:** A. Employment/Salary (full or part-time):; Kyungbook National University.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: OHRI start-up funding

Title: Atypical PKC-CBP pathway regulates murine adult neurogenesis

Authors: ***J. WANG**[1], K. HSU[1], L. HE[2], F. WONDISFORD[2], F. MILLER[3]
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Abstract: Our previous studies showed that CBP phosphorylation at serine 436 (S436) by atypical PKC (aPKC) is important for CBP to promote embryonic neural precursor differentiation in culture (Dev Cell, 2010). Recently, intriguing findings that CBP levels and/or activity are also required to regulate adult neurogenesis led us to ask whether aPKC-mediated phosphorylation of CBP at S436 is also a key modulator of adult neurogenesis. To do this, we used two knock-in mouse models to target the aPKC-CBP pathway, CBPS436A and p300G422S, where an aPKC phosphorylation site was modified in CBP and p300 alleles in order to generate phosphorylation-defective (CBPS436A) and phosphorylation-competent (p300G422S) mouse models. By using BrdU *in vivo* labeling technique, we showed that the total number of hippocampal newborn neurons (BrdU/NeuN+ cells) was significantly decreased in the CBPS436A mutants, while the total number of hippocampal newborn neurons (BrdU/NeuN+ cells) was significantly increased in the p300G422S mutants at the age of 3 months. More interestingly, we observed that the proportion of double labeled BrdU/NeuN + neurons over total BrdU + cells was only reduced in the hippocampi of CBPS436A mutants at the age of 6-month, associated with an increased proportion of double labeled BrdU/Sox2 + neural stem cells (NSCs)

in the same hippocampi. However, the total number of immature doublecortin (DCX) + neurons was not changed in the CBPS436A mutants at the age of 6 months. These data suggest that aPKC-mediated CBP phosphorylation is important for adult neurogenesis, and that it modulates differentiation of NSCs and maturation of newly-born neurons in an age-dependent fashion. In addition, our co-immunoprecipitation experiments showed that the association of CBP with CREB in the CBPS436A hippocampal tissues was abolished at the age of 6 months, but not at the age of 3-month, suggesting that CREB might be a downstream signaling of the aPKC-CBP pathway to regulate adult hippocampal neurogenesis in an age-dependent manner. To probe the upstream kinase that regulates the aPKC-CBP pathway to promote adult neurogenesis, we cultured subventricular zone (SVZ) adult neurospheres and showed that AMPK activators (metformin and AICAR) promoted adult neurogenesis in culture and that the increased neurogenesis caused by AMPK activators was abolished in cultures derived from CBPS436A mice, but potentiated in cultures derived from p300G422S mice. In summary, the aPKC-CBP pathway regulates adult hippocampal neurogenesis in an age-dependent manner and the aPKC-CBP pathway is essential to mediate AMPK-induced adult neurogenesis in culture.

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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Title: Neuroligin-1 knockdown reduces survival of adult-generated newborn hippocampal neurons *in vivo*

Authors: *E. SCHNELL[1], T. H. LONG[2], A. L. BENSEN[3], E. K. WASHBURN[3], G. L. WESTBROOK[3]
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Abstract: Adult-born hippocampal granule cells mature *in situ* over several weeks through the elaboration of dendrites and the formation of synaptic contacts. Survival of these adult-born neurons is modulated by neural activity, and thought to be enhanced by excitatory synaptic signaling. Using retrovirus-mediated gene knockdown in young adult mice *in vivo*, we confirmed that a reduction in expression of the synaptogenic protein neuroligin-1 decreases dendritic spine formation by immature newborn neurons in the dentate gyrus. At an early post-mitotic stage (21 days post-mitosis), the decrease in spines preferentially resulted from a decrease in filopodial-like spines without a change in excitatory synaptic transmission, suggesting that these filopodial-like structures were non-synaptic. Although primarily associated with synaptogenesis, neuroligin-1 knockdown during early granule cell maturation also delayed dendritic outgrowth, resulting in a decrease in dendritic branch number and length at 2 weeks post-mitosis. Neuroligin-1 knockdown was also associated with a reduction in long-term survival of newborn granule cells. Thus, our results indicate that neuroligin-1 not only has a role in dendritic spine formation during adult neurogenesis, but also has broader roles - in dendritic growth and the survival of newborn cells. We hypothesize that proper morphologic development of newborn cells is a prerequisite survival signal for newborn granule cells.

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Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: NIH R01 WYS0022AGG

Title: Self-regulation of adult hippocampal stem cells via secreted VEGF

Authors: *E. D. KIRBY, A. KUWAHARA, T. WYSS-CORAY
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Abstract: Vascular endothelial growth factor (VEGF) supports a number of potentially beneficial processes in the adult brain, including angiogenesis, synaptic plasticity cell proliferation, and neuronal survival. Though mature astrocytes were previously believed to be the primary source of VEGF within the brain, we have recently shown that adult neural stem/progenitor cells (NPCs) in the adult hippocampus secrete substantial amounts of VEGF, potentially helping to shape their own neurogenic niche. A number of cancer cell types similarly secrete VEGF and it has been hypothesized that this self-derived VEGF is an essential stem cell factor that supports the multipotent self-renewal of tumor cells. We therefore investigated whether self-secreted VEGF in adult hippocampal NPCs may similarly be stem cell essential. We found that mouse adult hippocampal NPCs express several isoforms of secreted VEGF as well as VEGFR2 (aka Flk1/KDR). Expression of both VEGF protein and its receptor were tightly regulated by growth factors and decreased dramatically with differentiation. Inhibition of VEGF via shRNA severely limited the long-term renewability of NPCs. In addition, transiently blocking VEGF signaling with either of two VEGFR2-specific kinase inhibitors reduced the number of recoverable large spheres in a sphere assay. Combined, our findings suggest that self-secreted VEGF in adult hippocampal NPCs is essential for maintaining stem cell identity and loss of VEGF may play a role in inducing differentiation. Current research is investigating the consequences of this stem cell self-regulation via VEGF *in vivo*, as well as the effects of NPC-derived VEGF on other cells in the neurogenic niche.

Disclosures: E.D. Kirby: None. **A. Kuwahara:** None. **T. Wyss-Coray:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

Support: SNSF Grant 31003A_153276

Title: GABA bidirectionally controls AP firing in newborn hippocampal granule cells

Authors: *S. HEIGELE, J. BISCHOFBERGER
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Abstract: Newly generated young hippocampal granule cells in the adult brain receive depolarizing GABAergic synaptic inputs, which were shown to be important for their development and functional maturation. Whether activation of GABAergic synapses can evoke action potential (AP) firing in newly generated granule cells is yet unknown. To identify the young neurons in the adult brain, we used transgenic mice expressing the red fluorescent protein DsRed under the control of the doublecortin (DCX)-promoter. Perforated-patch recordings (gramicidin) revealed that the reversal potential of GABAergic synaptic currents is substantially more positive in DCX-expressing young neurons (-34.2±2.1mV) as compared to mature granule cells (-71.9±2.9mV). Coincidentally activated with depolarizing current injections or glutamatergic synaptic transmission, GABAergic synapses generated a biphasic response pattern. GABAergic synaptic currents were indeed able to excite AP firing in young granule cells within a conductance window between ~0.5 and 6nS. Larger GABAergic inputs, however, effectively blocked AP firing via shunting inhibition, which might be important to protect the young cells from over excitation. Synaptic GABAergic transmission was fully blocked by 10µM gabazine, whereas a half maximal concentration (0.2µM) increased AP firing at high stimulation intensities, showing that both AP boosting and shunting inhibition are mediated by a GABA-A receptor mediated chloride conductance. Taken together, we show that GABAergic synaptic inputs in newly generated young granule cells can dynamically support either AP generation or shunting inhibition dependent on hippocampal network activity. Supported by Swiss National Science Foundation (SNSF)

Disclosures: **S. Heigele:** None. **J. Bischofberger:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Canadian Institutes of Health Research (A.S.)

National Sciences and Engineering Research Council of Canada (D.C.L.)

Title: The requirement of autophagy-related gene 5 (atg5) for adult hippocampal neurogenesis

Authors: *J. DHALIWAL[1], Y. XI[1], M. CEIZAR[2], M. VACULIK[1], K. L. KUMAR[2], M. SNAPYAN[3], A. SAGHATELYAN[3,4], D. C. LAGACE[1]
[1]Cell. and Mol. Medicine, Neurosci., [2]Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [3]Cell. Neurobio. Unit, Ctr. de recherche Inst. universitaire en santé mentale de Québec, Quebec city, QC, Canada; [4]Dept. of Psychiatry and Neurosci., Univ. Laval, Quebec City, QC, Canada

Abstract: Autophagy is an evolutionarily conserved lysosomal degradation pathway that contributes to the maintenance of cellular homeostasis. In stem cells, autophagy has cell type specific roles in cell maintenance, expansion and differentiation. The function of autophagy throughout the development of adult-generated neurons formed from neural stem and progenitor cells (NSPCs) remains unknown. Here we demonstrate autophagic flux in the NSPCs in the subgranular zone of the dentate gyrus and investigate the role of autophagy in adult neurogenesis through removal of the autophagy-related gene 5 (*Atg5),* which is required for the formation of autophagosomes. Using a tandem DsRed-LC3-GFP retrovirus we show autolysosomes are present in NSPCs throughout their development. Inducible removal of Atg5 in the adult brain using Glast-CreER^{T2} mice (6 weeks old) reduced the number of stem cells (GFP+Sox2+GFAP+) with a subsequent reduction in the number of dividing (GFP+Ki67+) as well as a decrease in the number of new neurons formed (GFP+DCX+NeuN+). These results suggest that Atg5 reduces the stem cell population and subsequently reduces neurogenesis in the absence of altering the fate of the NSPCs. To examine if Atg5 has a role in the survival of neural progenitor cells independent of its role in stem cells, we performed retroviral mediated ablation of Atg5 in the dividing progenitor cells in the dentate. At 7, 30 and 60 days post retroviral injection there was a significant reduction in the number of Atg5-null progenitor cells suggesting Atg5 is required for progenitor cell survival. The reduced survival of *Atg5*-deficient progenitor cells was rescued in mice that lacked the essential pro-apoptotic protein *Bax* (Bcl-2–associated X *protein)* supporting that *Atg-5* deficient cells die through a *Bax*-dependent mechanism. Additionally, removal of *Atg5* prevented running-induced increases in neurogenesis, supporting that autophagy contributes to the beneficial neurogenic effects of exercise. These findings highlight *Atg5* as an intrinsic regulator of NSPCs and regulator of adult neurogenesis.

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Poster

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Topic: A.03. Postnatal Neurogenesis

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Title: The role of epigenetic repression in a developmental sex difference in hippocampal neurogenesis

Authors: *S. L. STOCKMAN[1,2], M. M. MCCARTHY[1,2,3]
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Abstract: Sexual differentiation of the brain is an important process in normal development and has vast implications on later behavior. Our laboratory has identified a sex difference in neonatal hippocampal proliferation. Newborn male rats exhibit an increased rate of cellular proliferation in the dentate gyrus, CA1 and CA3 of the hippocampus compared to females and most of these become neurons. Administration of exogenous estradiol to newborn females increases cell genesis to levels consistent with males, whereas inhibition of estradiol signaling in males eliminates the sex difference (Bowers et al., Biol. Sex Diff., 2010). Sex differences are traditionally accomplished through elevation of testosterone in neonatal males that is locally aromatized to estradiol in the brain. However, throughout the period of increased male neurogenesis, there is no sex difference in hippocampal estradiol content implicating an alternative source of the difference (Konkle et. al, Endocrinology, 2011). Therefore, we hypothesized that suppression of estradiol mediated neurogenesis in females may be responsible for the observed sex difference in neonatal hippocampal proliferation. Epigenetic modifications translate environmental variables into enduring, but malleable, changes in gene expression making them appealing targets to mediate the sex differences seen in proliferation. Canonical modes of epigenetic regulation include direct modification of the DNA, primarily through methylation, as well as alteration to histone tails that modify the state of chromatin. The purpose of this study was to assess contributions of variations in histone acetylation in epigenetic repression of neurogenesis in females. On PN1 and PN2, male and female Sprague-Dawley rat pups were injected i.p. with the histone deacetylase (HDAC) inhibitor Trichostatin A (0.25mg/kg in 5% DMSO in saline) or comparative control and injected s.c. with either estradiol benzoate (100 ug/0.1 mL in sesame oil) or vehicle. Three hours after drug and hormone injection animals were injected with 5-bromo-2'-deoxyuridine (BrdU, 100mg/kg in saline). On PN3 animals were transcardially perfused, and brains sectioned coronally for BrdU immunohistochemistry. BrdU cell counts were conducted stereologically using StereoImager. Our results indicate that treatment of newborn females with HDAC inhibitors mirror the increase in proliferation seen following administration of exogenous estradiol (ANOVA, $p<0.05$). These findings suggest that

exogenous estradiol may act to increase total histone acetylation to overcome suppression of estradiol mediated neurogenesis in females. Supported by NIH grant R01NS 050525 to MMM

Disclosures: S.L. Stockman: None. **M.M. McCarthy:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 394.26/A26

Topic: A.03. Postnatal Neurogenesis

Title: Brain-specific expression of lysosomal-associated membrane protein 5 (LAMP5) gene

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Abstract: In the mammalian central nervous system (CNS) more than a half of the genes in the genome are expressed in a spatiotemporally regulated manner. Although they are thought to be involved in brain developments and functions, most of them remain to be studied for the subcellular localizations and molecular functions of their encoded proteins in the CNS. When they are expressed in a specific stage of the brain development or in a specific cell type or brain region, the genes should be involved in the neural development and function, such as differentiation of neurons, neural network formation, and synaptic plasticity. Recently, we have developed Cerebellar Development Transcriptome Database (CDT-DB) (Sato et al., Neural Netw, 2008), and identified several brain-specific genes whose encoded proteins have not yet been characterized for their functional significance in the CNS including the gene encoding lysosomal-associated membrane protein 5 (LAMP5, also known as BAD-LAMP). We found that Lamp5 is highly expressed in embryonic mouse cerebellum and is down-regulated in later developmental stages while its expression at the whole brain level increases along with the development. The nematode orthologous gene of Lamp5, unc-46, encodes a type III transmembrane protein with several glycosylation sites. UNC-46 has been shown to locate in the synaptic terminal of GABAergic neurons and regulate the release of GABA, an inhibitory neurotransmitter that plays an important role in brain development. A previous study also showed that LAMP5 protein was expressed in the II/III and V layers of mouse postnatal cerebral cortex. However, its expression over other brain regions during developmental stages is still unknown. In the present study, we have performed immunohistochemistry of murine brain

sections at various developmental stages with a polyclonal antibody against LAMP5 and found its expression in cerebellum and a certain cell population, which is presumably located near the locus coeruleus. We are now generating a Lamp5 knockout mouse to investigate the physiological function of LAMP5 in the CNS.

Disclosures: **M. Koebis:** None. **Y. Saito:** None. **Y. Shinoda:** None. **T. Furuichi:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Title: Effects of TRPV1 deficiency on the adult hippocampal neurogenesis

Authors: ***S.-E. WANG**[1], S. KO[1], H. JO[1], H. SON[1,2]
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Abstract: Transient Receptor Potential family Vanilloid 1 (TRPV1) is well known for stress response protein in the central nervous system. Activation of TRPV1 polymodal cation channels permits the Ca^{2+} influx through it. Ca^{2+} influx through TRPV1 can induce endocytosis of AMPAR that results in long-term depression (LTD) and neurodegeneration. Since intracelluar increase of Ca^{2+} regulates synaptic plasticity and signal transduction in neurons, we first hypothesized that TRPV1 deficiency may affect neuronal plasticity including adult hippocampal neurogenesis. We show that the number of 5-bromo-2'-deoxyuridine (BrdU)(+) cells is increased in the hippocampal dentate gyrus (DG) of TRPV1 KO mice compared to wild type C57B/L6. Furthermore, expression of double cortin (DCX) and polysialylated-neural cell adhesion molecule (PSA-NCAM), which are markers of early neuronal differentiation, are enhanced in hippocampal DG of KO mice compared to wild type mice. Loss of TRPV1 also increases synaptic molecules, such as postsynaptic density-95 (PSD95), glutamate receptor 1 (GluR1) and synapsin1. These results suggest that TRPV1 deficiency may positively regulate neuronal plasticity including adult hippocampal neurogenesis. *Keywords ; TRPV1, neurogenesis*

Disclosures: **S. Wang:** None. **S. Ko:** None. **H. Jo:** None. **H. Son:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Program#/Poster#: 394.28/A28

Topic: A.03. Postnatal Neurogenesis

Title: Delayed cerebellar development in transgenic mouse expressing a mutant thyroid hormone receptorβ-1 in Purkinje cells

Authors: L. YU, T. IWASAKI, N. SHIMOKAWA, *N. KOIBUCHI
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Abstract: Thyroid hormone (TH) is essential for growth and development of cerebellum. TH exerts its major roles by binding to TH receptors (TRs). Deficiency of TH during perinatal period causes abnormal brain development. To study the role of TH on cerebellar development, we have generated a transgenic mouse expressing a dominant-negative TR specifically in the cerebellar Purkinje cell. A mutant human TRβ1 (G345R), which binds to TH-response element but cannot bind to T3, was subcloned into exon 4 of the full length L7/ Pcp-2 gene, which is specifically expressed in the Purkinje and the retinal rod bipolar cells. The transgene was specifically expressed in the Purkinje cell in the postnatal cerebellum. Postnatal Purkinje cell dendritic arborization was significantly delayed in the transgenic mice. To our surprise, granule cell migration was also significantly delayed. In the primary cerebellar culture, TH-induced Purkinje cell dendrite arborization was also suppressed. Using semi-quantitative PCR, we have examined the change in TH-responsive gene mRNA levels. Levels of IP3 receptor type1 and RORα mRNA, which are mainly expressed in the Purkinje cell, and BDNF mRNA, which is expressed in both Purkinje and granule cells were significantly decreased. Furthermore, levels of NT-3 mRNA, which is mainly expressed in the granule cell was also decreased. Levels of myelin basic protein mRNA, which is mainly expressed in the oligodendrocyte was not altered. Motor coordination of transgenic mice was significantly disrupted. These results indicate that the development of Purkinje cell is directly regulated by TH through binding to TR, whereas TH action through TR in the Purkinje cell is also important for development of other subset of cerebellar cells such as granule cells.

Disclosures: L. Yu: None. **N. Koibuchi:** None. **T. Iwasaki:** None. **N. Shimokawa:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

Support: Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009521).

Title: Novel osmotin attenuates glutamate-induced synaptic dysfunction and neurodegeneration via the JNK/PI3K/Akt pathway in postnatal rat brain

Authors: G. YOON, S. SHAH, *M.-O. KIM
., Dept. of Biol., Gyeongsang Natl. Univ., GAZA 900, Jinju, Korea, Republic of

Abstract: Overactivation of the glutamatergic-induced excitotoxicity pathway has been reported in several neurodegenerative diseases. Molecules that inhibit the release of glutamate or the over-activation of glutamate receptors can minimize neuronal cell death in these diseases. Osmotin, a homologue of mammalian adiponectin, is a plant protein has been examined in the present study for the first time to determine how it may protect against glutamate-induced synaptic dysfunction and neurodegeneration in the postnatal day seven rat brain. The results indicate that glutamate treatment induced excitotoxicity by over activating glutamate receptors, synaptic dysfunction and neuronal apoptosis after four hours in the cortex and hippocampus of the postnatal brain. On the other hand, post administration of osmotin, significantly reversed glutamate receptors activation, synaptic deficit and neuronal apoptosis by stimulating the JNK/PI3K/Akt intracellular signaling pathway. Moreover, osmotin treatment also abrogated glutamate-induced DNA damage, apoptotic cell death and attenuated the localization and distribution of p53 and p-Akt and caspase-3 in the hippocampus of postnatal brain. Taken together all these results suggest that osmotin might be a novel neuroprotective agent in the excitotoxic diseases.

Disclosures: G. Yoon: None. **S. Shah:** None. **M. Kim:** None.

Poster

394. Postnatal Neurogenesis: Molecular Mechanisms

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Topic: A.03. Postnatal Neurogenesis

Support: Manitoba Health Research Council (MHRC)

Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Corticogenesis of the cerebellar cortex in lysosomal acid phosphatase (Acp2) mutant mice

Authors: *H. MARZBAN[1], K. BAILEY[1], M. RAHIMI BALAEI[1], A. U.MANNAN[3], S. GHAVAMI[2]
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Abstract: Introduction: The cerebellum is a highly organized centre of motor coordination and cognition. Structurally it consists of a three-layered cortex with distinct neuronal subtypes, such as granule cells (gcs) and Purkinje cells (Pcs). It is suggested that Pcs utilize reelin dependent pathways to form a monolayer in the cerebellar cortex. Reelin expressed by gcs, is required for Pcs distribution from the clusteric stage to establish a monolayer of Pcs between the molecular and granular layers of the cerebellar cortex. Proliferation of the gcs is influenced by Shh (Sonic hedgehog) expressed in Pcs. A mouse mutant called nax (naked-ataxia), resulting from a spontaneous mutation in lysosomal acid phosphatase (Acp2), presents multi-layered Pcs that ectopically invade the molecular layer. We hypothesize that the establishment of this mono layered Pcs is not dependent on the reelin pathway. Materials and Methods; Acp2 mutant mice were used for this study. Molecular expression and distribution were assessed by immunohistochemistry and Western blotting. Results; The cerebellar cortex of the Acp2 mutant mice which was characterized by the absence of the vermis, reveals the presence of Pcs in a randomized, dispersed manner spanning the entire molecular layer rather than a monolayer in the cerebellar cortex. The amount of gcs is severely reduced in the cerebellum which is more prominent rostrally as compared to their numbers in the caudal cerebellum. It is also observed that Pax6 expression follows the pattern of gcs proliferation and migration during postnatal development. The pattern of reelin expression is down-regulated in the Acp2 mutant cerebellum at around P12 and accompanies the down-regulation of Shh. Conclusion; The down-regulation of Shh that followed the declined reelin expression may be secondary to Pcs degeneration. However, the presence of reelin is comparable with wild type during early postnatal development, indicative of reelin effect during clustric stages however failed to form mono layered Pcs. Pcs differentiation is severely delayed inthe Acp2 mutant cerebellar cortex and it can be concluded that multilayer Pcs may be due to the failure of appropriate cross-talk between Shh and the reelin signalling pathway during postnatal cerebellar development.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: FAPESP

Title: Differences in gender response at the hippocampal neurogenesis following nociceptive stimulus and fentanyl in newborn rats

Authors: ***A. T. LESLIE**[1], D. S. BANDEIRA[2], C. SILVA[2], D. S. ENGELKE[2], R. GUINSBURG[1], L. E. A. MELLO[2]
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Abstract: There is growing evidence that untreated pain experienced in the neonatal period could lead to changes in the neuronal circuitry, which may be associated to an adverse neurodevelopmental outcome. Moreover, pain treatment with opioid is not free of deleterious effects on the central nervous system. In this study we examined the effect of the neonatal inflammatory pain, initiated in the postnatal day 1 of life (PN1), followed or not by analgesia, over the hippocampal neurogenesis in a rat model. Female and male Wistar rats were randomly assigned to four groups: control (without any stimulus); pain, whereas the nociceptive inflammatory stimulus was performed by the injection of the complete freund´s adjuvant (CFA) into the plantar surface of the paw on postnatal day 1; fentanyl (daily injections from PN1-PN8); and analgesia, fentanyl injection 30 minutes before the CFA injection on PN1, followed by daily fentanyl injection from PN2 to PN8. On PN9, 5-bromo-20-deoxyuridine (BrdU) was given, and 24h later the animals were perfused. BrdU+ cells were counted in the subgranular zone of the dentate gyrus of the hippocampus. Kruskal-Wallis was applied, being significant $p<0.05$. We found that the male analgesia group showed greater number of BrdU+ cells compared with the male fentanyl group (p .0005). The female analgesia group also presented with more BrdU+ cells in the dentate gyrus compared to the fentanyl group. Our findings suggested differences at the hippocampal neurogenesis among the male and female rats, following an inflammatory painful stimulus and the fentanyl administration, represented by an increase of the neurogenesis with

CFA and fentanyl administrations. We are still working on more data looking for the explanation for these results.

Number of BrdU+ cells in the dentate gyrus of the hippocampus		
Group	Mean	N
Female Control	8.1	4
Male Control	13.1	4
Female Pain	9.3	4
Male Pain	12.3	4
Female Analgesia	54.6	4
Male Analgesia	52.1	4
Female Fentanyl	20.1	4
Male Fentanyl	7.1	4
BrdU+ cells are expressed by total number of BrdU+ cells/the length of dentate gyrus (μm) multiplied		

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: Halls A-C

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Program#/Poster#: 395.02/A32

Topic: A.03. Postnatal Neurogenesis

Title: High aerobic capacity is necessary and sufficient for the beneficial effects of exercise on hippocampal neurogenesis and cognition

Authors: *C. M. TOGNONI[1], J. M. SAIKIA[1], J. DU[1], K. M. ANDREJKO[1], E. A. BABB[2], R. M. PEACE[3], L. G. KOCH[4], S. L. BRITTON[4], L. W. JONES[5], C. L. WILLIAMS[1]
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Abstract: It is commonly accepted that exercise (e.g., wheel running or treadmill training) increases hippocampal neurogenesis and cognitive function. However, exercise exerts myriad effects on body and brain, and it is unclear what the contribution of aerobic capacity, the body's ability to consume oxygen, is to these effects. To isolate the role of aerobic capacity in hippocampal plasticity, we exploited selectively-bred rat models of acquired (high/low responders to training: HRT/LRT rats) and inherent (high/low capacity runners: HCR/LCR rats) aerobic capacity. HRT and LRT rats have the same aerobic capacity at baseline, yet after 8 wks of comparable treadmill training, HRT but not LRT rats significantly increase their aerobic capacity. While sedentary LRT and HRT rats had equivalent levels of hippocampal neurogenesis, only HRT rats had increased DCX+ young neurons following training. These data were positively correlated with their performance on pattern separation tasks, which have been shown to be neurogenesis-dependent. Increased aerobic capacity appears to be necessary for exercise to increase in hippocampal neurogenesis and cognitive function. To determine whether high aerobic capacity is sufficient for enhanced hippocampal function, we examined HCR and LCR rats, which have an inherent 2- to 3-fold difference in aerobic capacity. We found that HCR rats also had 2- to 3-fold more DCX+ young neurons in the hippocampus than LCR rats and rats from the heterogeneous founder population. HCR rats also had 4- to 5- fold more newborn BrdU+ cells that had matured into NeuN+ adult neurons compared to LCR rats, but no differences in the number of BrdU+ cells 24 hr after injection, indicating that aerobic capacity has a specific effect on neuronal survival in the hippocampus. In contrast, when neurogenesis in the subventricular zone was examined, HCR rats had more proliferation in this region than LCR rats but did not appear to have more surviving neurons in the olfactory bulb. We asked whether HCR's enhanced neurogenesis translated to enhanced hippocampal cognition, as is typically seen in exercise-trained animals. Compared to LCR rats, HCR rats performed with higher accuracy on pattern separation tasks, and their performance was positively correlated with their levels of hippocampal neurogenesis. Together, these findings demonstrate that aerobic capacity, not exercise *per se*, is critical for enhanced hippocampal plasticity and function. Because human populations are widely variable in inherent and acquired aerobic fitness, these findings have important implications for the recommendation of physical exercise as a cognitive therapy.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: NIH Grant DC011137

Title: Patterns of olfactory bulb cell genesis are altered with long-term, partial deafferentation in adult zebrafish

Authors: D. M. TRIMPE, *C. A. BYRD
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Abstract: Our lab has shown that repeated intranasal irrigation with Triton X-100 in adult zebrafish severely alters the olfactory organ and diminishes innervation to the olfactory bulb, resulting in a decrease in bulb volume. Cessation of the treatment allows for reinnervation and recovery of bulb volume. We predicted that patterns of cell genesis could account for deafferentation-induced bulb volume reduction and reafferentation-induced bulb volume recovery. The hypothesis of this study was that the decrease in bulb size, seen after reduced afferent input with repeated detergent ablation of the olfactory epithelium, is due to decreased cell genesis or migration of newly formed cells into the bulb and that cessation of the detergent treatment will result in a reversal of the deafferentation-induced reduction in bulb size through increased cell genesis or migration into the olfactory bulb. First, potential effects on proliferation in the ventricular zone were examined in 3-week chronically Triton X-100-treated fish and in fish allowed to recover for 3 weeks using anti-bromodeoxyuridine, anti-proliferating cell nuclear antigen, and anti-SOX2. Fish were exposed to bromodeoxyuridine via intraperitoneal injection. Cell proliferation in the olfactory bulb was not affected by this deafferentation or by recovery. Additionally, preliminary data showed no observable differences in proliferation in the ventricular zone. Next, migration of newly formed cells into the olfactory bulb was examined by exposing fish to bromodeoxyuridine at the time of the initiation of Triton X-100 treatment, followed by chronic detergent treatment every three days for three weeks. Contrary to our hypothesis, we observed a bilateral increase in anti-bromodeoxyuridine labeled profiles in the olfactory bulb ($P<0.05$ for the treated bulb and $P<0.05$ for the internal control bulb). Allowing the bulb to recover from detergent treatment for three weeks did not reduce this bilateral increase. These findings suggest that recovery of bulb volume after chronic detergent treatment may be due, at least in part, to increased migration of cells into the olfactory bulb internal cell

layer. These studies permit investigation into the adult brain's potential for recovery from physical or functional deafferentation due to injury or disease.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: A2 Corporation Research Grant

Title: Gluten and casein-derived opiate peptides alter redox status and produce epigenetic-based differences in gene expression

Authors: *N. HODGSON[1], M. TRIVEDI[1], R. DETH[1], A. CLARKE[2]
[1]Pharmaceut. Sci., Northeastern Univ., Boston, MA; [2]A2 Corp. Ltd, Auckland, New Zealand

Abstract: Intolerance to wheat and milk causes symptoms of gastrointestinal (GI) inflammation in sensitive individuals and the prevalence of this intolerance is increasing. GI inflammation can adversely affect the absorption of critical nutrients and can also affect immune cells, which are resident in the intestine. Gluten and casein are prominent proteins from wheat and milk, respectively, and their intestinal hydrolysis gives rise to homologous seven amino acid peptides with opiate activity. A gluten-free/casein-free (GF/CF) diet can relieve symptoms of GI inflammation and is also reported to improve neurological conditions including autism and schizophrenia, as well as reducing autoimmunity. Since oxidative stress and impaired methylation have been implicated in autism and schizophrenia, we examined the influence of gluten- (GM7) and both human and bovine and casein-derived opiate peptides (hBCM7 and bBCM7), on redox and methylation status in cultured SH-SY5Y neuroblastoma cells. All three peptides caused time-dependent changes in cysteine uptake, and levels of cellular metabolites in glutathione synthesis (antioxidant) and methionine cycle (methylation) pathways, similar to, but less extensive than, the effects of morphine. After a 4 hr treatment with hBCM7, bBCM7 or morphine we observed changes in the global pattern of DNA methylation, using MBD-SEQ to identify site-specific changes in CpG methylation. Differential effects of hBCM7 vs. bBCM7 on promoter methylation status could be discerned. Microarray studies revealed differential gene transcription associated with the changes in global DNA methylation. Morphine, hBCM and

bBCM treatment showed both overlapping changes in transcription as well as changes which were distinct for each agent. The changes induced by bovine form of BCM7 were similar to morphine and much different to the human form. This study is the first study to link casein/gluten derived peptides to epigenetic changes in neurological disorders, and provides a novel mechanistic explanation for the benefit of GF/CF dietary intervention for the treatment of autism and other inflammatory disorders. It is noteworthy to mention that, BCM7 is released only from the A1 type of casein and not the A2-beta casein.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Title: Identification of the metabolic fuel requirements of adult neural stem cells

Authors: *E. STOLL[1], R. MAKIN[2], I. R. SWEET[5], A. TREVELYAN[3], S. MIWA[4], P. J. HORNER[6], D. M. TURNBULL[3]
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Abstract: Neural activity is tightly coupled to energy consumption, particularly sugars such as glucose. Yet we find that, unlike mature neurons and astrocytes, neural stem/progenitor cells (NSPCs) do not require glucose to sustain aerobic respiration. NSPCs within the adult subventricular zone express enzymes required for fatty acid oxidation and show sustained increases in oxygen consumption upon treatment with a polyunsaturated fatty acid. NSPCs also demonstrate sustained decreases in oxygen consumption upon treatment with etomoxir, an inhibitor of fatty acid oxidation. In addition, etomoxir decreases the proliferation of subventricular zone NSPCs without affecting cellular survival. Finally, higher levels of neurogenesis can be achieved in aged mice by ectopically expressing PGC1a, a factor that increases cellular aerobic capacity by promoting mitochondrial biogenesis and metabolic gene transcription. Endogenous neural stem cells are excellent therapeutic targets, retaining the capacity to rebuild and repair tissue in the adult brain by producing new neurons and astrocytes. Since catabolic activity is a fundamental characteristic of a cell, exquisitely optimized to meet energetic needs and constraints, the identification of metabolic substrates required by the adult neural stem cell is imperative to understanding the process of regeneration. Regulation of metabolic fuel availability could prove a powerful tool in promoting cellular proliferation in the central nervous system. Boosting the regenerative potential of the aging brain by providing additional aerobic respiratory capacity may alleviate cognitive deficits associated with normal aging, such as impairments in working memory and olfaction.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

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National Brain Tumor Society

Title: Effects of intranasal epidermal growth factor treatment on the subventricular zone after chronic perinatal hypoxia

Authors: *J. SCAFIDI[1], J. EDWARDS[2], J. KURZ[3], V. GALLO[2]
[1]Dept of Neurol. and Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., WASHINGTON, DC; [2]Ctr. for Neurosci. Res., [3]Dept of Neurol., Children's Natl. Med. Ctr., Washington, DC

Abstract: There are no effective treatments available that improve function in the growing population of very preterm infants (<32 weeks gestation) with neonatal brain injury. Diffuse white matter injury (DWMI) is a common finding in these children that contributes to their chronic neurodevelopmental impairment. As we recently published (Scafidi et al., Nature, 2014), using chronic perinatal hypoxia (postnatal day P3-11) as a clinically relevant mouse model of very preterm brain injury, the administration of intranasal epidermal growth factor (HB-EGF; P11-14) stimulates the endogenous response of EGF receptor (EGFR)-expressing progenitor cells in the white matter and promotes cellular, biochemical and functional recovery. It is established that injury results in a significant expansion of the neural stem cells (NSCs) and progenitor cells that reside in the subventricular zone (SVZ). However, it is unknown whether administration of intranasal HB-EGF in the developing brain provides an additive stimulatory effect on newly generated NSCs and progenitor cells after chronic perinatal hypoxia and the specific phenotype of the cells that results from HB-EGF treatment *in vivo*. In this study, we demonstrate the following results in the SVZ after chronic hypoxia: i) a significant increase in phosphorylated-EGFR and EGFR ligand expression immediately after hypoxia but not at later time points; ii) a significant increase in the number of proliferating cells (anti-Ki67+); iii) a significant increase in number of Ascl1-expressing bipotential progenitor cells; and iv) a significant increase in the number of NG2-expressing oligodendrocyte progenitor cells (OPCs). Interestingly, there was a significantly additive, but transient (P15, but not P18) effect on all cell types in the chronic hypoxia group treated with intranasal HB-EGF, while inhibition of EGFR using an antagonist prevented any hypoxia-induced increases in these cell populations. Finally, we also used fate-mapping techniques with tamoxifen-inducible Cre-transgenic mice to demonstrate the effects of chronic hypoxia and HB-EGF on SVZ GFAP-expressing stem cells and PDGFalphaR-expressing progenitor cells. Our results indicate that hypoxia promotes expansion of SVZ progenitor cells and HB-EGF potentiates the effects of the injury. Therefore, understanding the effects of intranasal HB-EGF treatment on the SVZ is crucial in order to develop this treatment as a potential therapeutic strategy for neonatal brain injury arising from very premature birth.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: CIHR grant MOP#285993

CBCF#10470

Title: Physical exercise prevents suppression of hippocampal neurogenesis and mitigates cognitive impairment in chemotherapy-treated rats

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Abstract: Increasing evidence shows chemotherapy-induced cognitive impairments in humans and rodents. These deficits include confusion, memory loss, decreased attention span, and inability to focus or concentrate. Physical exercise is known to enhance hippocampal neurogenesis and improve cognitive function. In this study we examined the effect of physical exercise on rats that were treated with chemotherapeutic agents. 3-month-old Long-Evans rats (n=37), housed in either standard cages or cages that allowed unlimited access to a running wheel, received intra-peritoneal injections of 5-fluorouracil and methotrexate, or equal volumes of saline. They subsequently underwent a series of cognitive tasks - including spatial memory (SM), non-matching-to-sample rule learning (NMTS), and delayed NMTS (DNMTS) tests. Expression levels of Ki67, doublecortin (DCX) were examined in the dentate gyrus. Analysis of variance (ANOVA) was used to test differences between groups on behavioral measures and cell counts. First, chemotherapy significantly reduced the number of DCX+ cells in the dentate gyrus by approximately 25% ($P=0.02$), while running markedly increased DCX+ cells ($P<0.01$). In addition, rats exposed to running showed evident increases in both Ki67+ ($P<0.05$) and BrdU+/DCX ($P<0.05$) cells. No significant difference in average running distance was observed between saline- and chemotherapy-treated rats ($P>0.25$). Behaviorally, ANOVAs showed significant main effects of chemotherapy on performance in SM, NMTS and DNMTS. Running improved the performance on all these tasks. In summary, we report promising results where the adverse effects of chemotherapy on both hippocampal neurogenesis and behavioral performance were rescued by running, indicating physical exercise as a highly feasible and safe therapeutic intervention against chemotherapy-induced cognitive deficits.

Disclosures: J. Huang: None. **G. Winocur:** None. **J.M. Wojtowicz:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 395.08/A38

Topic: A.03. Postnatal Neurogenesis

Title: Why social interaction can facilitate adult neurogenesis

Authors: A. M. DIOS, *M.-F. CHENG
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Abstract: We have previously shown that adult neurogenesis in the ventral medial nucleus of the hypothalamus (VMN) can be facilitated by social factors. When a male ring dove is housed with a female mate in comparison to being housed in isolation or with a male, there is a significantly higher level of mature new neurons in the VMN lesion area and an increase in courtship function. In this study, we seek to determine whether an increase in the production of new neurons in males can be explained by properties of the female stimulus or is a result of the male's own activity that engages the circuitry involved in neurogenesis. In study one, male doves were housed with females that they were bonded to versus those that they were not bonded to, namely, two similar stimuli that provoke varying levels of male behavior. The VMN was lesioned and the number of double labeled NeuN+/BrdU+ cells at the lesion site were analyzed using a confocal laser microscope immediately after lesion and at weeks 2,4, and 8 post lesion. Recovery of courtship behavior was measured during these time points. Behavior of all males and the females they were housed with were recorded every three days during the 8 week recovery period. Brain derived neurotrophic factor (BDNF) is closely associated with the neurogenesis process. In study two, we determine whether activity dependent BDNF level is associated with levels of neurogenesis by comparing BDNF levels in bonded versus non-bonded males (ie: males engaged in low versus high courtship activity).

Disclosures: **A.M. Dios:** None. **M. Cheng:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: NSERC discovery grant

CIHR operating grant (MOP:119271)

Title: Long-term effects of early exercise on adult hippocampal neurogenesis in aging rats

Authors: *C. M. MERKLEY, C. JIAN, A. MOSA, Y.-F. TAN, J. WOJTOWICZ
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Abstract: It is well established that early life experience is associated with cognitive ability in old age and the development of neurological disease. The Neurogenic Reserve hypothesis has emerged as a mechanism for cognitive reserve, based on evidence that enrichment maintains neurogenesis at a higher level and results in improved learning and memory. The majority of studies to date have examined short-term consequences of enhancing or blocking neurogenesis but long-term changes with age resulting from early intervention remain less well understood. The goal of this study was to address the hypothesis that early life experience, such as a period of voluntary running in juvenile rats, can alter properties of adult neurogenesis for the remainder of the animal's life. Briefly, male Long Evans rats were grouped into Runners (running wheel access) and Controls (sedentary). After a 30 day running period, cohorts of animals were sacrificed 1 week, 5 weeks, 6 months and 9 months post-running. All animals were injected with thymidine analogues, 5-iodo-2'-deoxyuridine (IdU) and 5-chloro-2'-deoxyuridine (CldU), 1 week and 4 weeks prior to perfusion, respectively. Single and dual-label immunohistochemistry for IdU and CldU was conducted along with other immunohistological markers to assess cellular proliferation (Ki67), neuronal differentiation (Doublecortin (DCX)), survival and maturation (Calbindin (CaBP)) of the labeled adult-born cells. Our results indicate that the number of differentiating neuronal precursors is increased in Runners early on, but within 5 weeks post-running has returned to Control levels. However, the rate of neuronal maturation and survival during a four week period after cell division was enhanced in rats up to 11 months of age (9 months post-running).These results suggest that short term enhancement of neuronal differentiation is under homeostatic regulation, whereas neuronal maturation and survival show long-term alterations with age that do not appear to be regulated in a homeostatic manner. This study is the first to show that a transient period of physical activity at a young age promotes changes in neurogenesis that persist over the long-term, which is important for our understanding of the modulation of neurogenesis by exercise with age. Functional integration of adult-born neurons within the hippocampus that resist homeostatic regulation with aging may be an essential feature of adult neurogenesis that promotes the maintenance of neural plasticity in old age.

Disclosures: **C.M. Merkley:** None. **C. Jian:** None. **A. Mosa:** None. **Y. Tan:** None. **J. Wojtowicz:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: NIH/NIDA DA016765

DA016765-07S1

DA023555

DA007290

Title: Reduction of adult hippocampal neurogenesis via cranial irradiation enhances morphine self-administration and morphine-induced locomotor sensitization

Authors: ***S. E. BULIN**[1], D. R. RICHARDSON[1], K. H. SONG[2], T. D. SOLBERG[2], A. J. EISCH[1]
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Abstract: Drugs of abuse dynamically regulate adult hippocampal neurogenesis, a process important for hippocampal-dependent functions such as context learning and memory, context discrimination, and regulation of the stress response. To test the hypothesis that hippocampal neurogenesis is involved in the vulnerability to morphine addiction, we assessed whether ablation of neurogenesis enhances vulnerability to addiction during morphine self-administration (MSA) and morphine locomotor sensitization (MLS). Male Sprague-Dawley rats (~6 weeks old) were exposed to either sham treatment (Sham) or image-guided cranial x-ray irradiation (IRR) to eliminate new hippocampal neurons. Ablation was confirmed via absence of doublecortin+ immature neurons. Six weeks post-IRR when inflammation subsided, rats began either MSA (Sham=16, IRR=15) or MLS (Sham=12, IRR=12). For MSA, IRR rats self-administered more morphine vs. Sham rats ($p<0.019$). This was not a general enhancement of learning, motivation, or locomotion, as operant learning and locomotor activity were unchanged. After 28 days of withdrawal, IRR rats exhibited higher context-induced reinstatement than Sham rats ($p<0.038$). In a separate group of rats used for MLS, IRR rats exhibited a dose-dependent enhancement of

morphine locomotor sensitization, with a greater increase in beam breaks vs. Sham rats when sensitized at 5 mg/kg ($p<0.018$), but similar increases at 10 mg/kg, suggesting a ceiling effect. Irradiated rats also showed increased activity during a dose-response experiment at 1 mg/kg. Along with previous studies, these data indicate that reduced hippocampal neurogenesis confers vulnerability for multiple classes of drugs of abuse. While other irradiation-induced changes (e.g. gliosis) are being considered for their involvement in these behaviors, it is intriguing to consider whether therapeutics that increase or stabilize neurogenesis prevent addiction and/or relapse. Mechanistic studies are also ongoing to explore the involvement of neurogenesis in the neural circuitry underlying the morphine-induced activation of the hippocampus and downstream brain areas, and how this circuitry is altered when adult neurogenesis is ablated.

Disclosures: **S.E. Bulin:** None. **K.H. Song:** None. **T.D. Solberg:** None. **D.R. Richardson:** None. **A.J. Eisch:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program#/Poster#: 395.11/A41

Topic: A.03. Postnatal Neurogenesis

Support: NSF Cooperative Agreement Award EPS-1003907

Title: Fructose impairs neuronal differentiation of adult neural stem cells *in vitro*

Authors: J. A. LEONARD[1], A. L. RAMIREZ GARCIA[1], *N. SPITZER[2]
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Abstract: In recent decades the consumption of fructose, as high fructose corn syrup in processed foods, has increased dramatically and has now been recognized as a significant health concern. In hepatocytes and adipocytes, high levels of fructose generate uric acid, leading to generation of reactive oxygen species (ROS) that cause cellular damage. Fructose penetrates the blood brain barrier through the Glut5 transporter, but its effects on the mechanisms operating in neurons is not well understood. In animals exposed to a high-fructose diet, cognitive deficits similar to those associated with aging are observed. Reduced neurogenesis is thought to contribute to the cognitive decline in aging, indeed, a high-fructose diet also reduces adult neurogenesis, although the cellular mechanisms by which this occurs is unclear. In mammals,

defined populations of adult neural stem cells (NSCs) are located in the hippocampus and the subventricular zone (SVZ). Here, they continue to proliferate, giving rise to progenitor cells that migrate, differentiate and integrate into existing circuitry where they are involved in plasticity with roles in memory formation, learning, behavioral responses, and reward systems. In addition, NSCs are thought to migrate to damaged brain tissues and contribute to repair. *In vivo* and *in vitro*, NSCs can differentiate into oligodendrocyte, astrocyte, or neuronal phenotypes in response to appropriate chemical signals. Adult NSCs from the SVZ can be maintained as progenitors in culture and plated in conditions that encourage differentiation, allowing investigation of the cellular mechanisms underlying this process at the level of individual cells. This accessible model system has been used to identify many of the exogenous signals that drive differentiation, and the intracellular mechanisms involved. We tested the effects of fructose exposure on the mechanisms underlying differentiation in NSCs cultured from the SVZ of young adult rats. Using immunocytochemistry, we found that the proportion of cells expressing β-tubulin III, an early neuron marker, was reduced in cells cultured in media containing fructose. This indicates that fewer NSCs differentiate towards a neuronal lineage after fructose exposure. Time lapse microscopy revealed that the neurites extended by NSCs cultured in the presence of fructose are less complex, with fewer branch points than those differentiating in control conditions. These results suggest that fructose inhibits the maturation of NSCs towards a neuronal fate. These cellular effects on differentiating NSCs could contribute to the cognitive decline observed in animals subjected to a high-fructose diet.

Disclosures: **J.A. Leonard:** None. **N. Spitzer:** None. **A.L. Ramirez Garcia:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: the Funding Program for the Next Generation World-Leading Researchers LS104

JSPS KAKENHI 23680041

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Title: Interferon-α inhibits neurogenesis and induces depression-like behavioral phenotype via interferon receptors expressed in the mouse brain

Authors: *N. KANEKO[1], L.-S. ZHENG[1,3], S. HITOSHI[4,5], K. TAKAO[5], T. MIYAKAWA[5,6,7], Y. TANAKA[2], U. KALINKE[8], K. KUDO[9], S. KANBA[10], K. IKENAKA[5], K. SAWAMOTO[1]
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Abstract: New neurons generated by the neural stem cells (NSCs) in the adult hippocampus play an important role in emotional regulation and respond to the action of antidepressants. Depression is a common and serious side effect of interferon-α (IFN-α), which limits its use as an antiviral and anti-tumor drug. However, the mechanism(s) underlying IFN-induced depression are largely unknown. We characterized the behavior of mice subjected to chronic IFN-α treatment using a comprehensive battery of behavioral tests, and found that the animals exhibited a depression-like behavioral phenotype including learned helplessness and decreased social interactions. Using these mice and a neuronal cell culture system, we found that IFN-α directly suppressed NSC proliferation, resulting in the reduced generation of new neurons. Both systemic and brain-specific mouse knockouts of the IFN-α receptor prevented IFN-α-induced depressive behaviors and the inhibition of neurogenesis, suggesting that IFN-α suppresses hippocampal neurogenesis via its receptor in the brain, to induce depression. These findings provide new insight for understanding the neuropathology underlying IFN-α-induced depression and for developing new strategies for the prevention and treatment of IFN-α-induced depressive effects.

Disclosures: **N. Kaneko:** None. **L. Zheng:** None. **S. Hitoshi:** None. **T. Miyakawa:** None. **Y. Tanaka:** None. **U. Kalinke:** None. **K. Kudo:** None. **S. Kanba:** None. **K. Ikenaka:** None. **K. Sawamoto:** None. **K. Takao:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: NIA/ NIH Intramural Research Program

Title: Exercise modifies the neuronal network of newborn dentate granule cells

Authors: *C. VIVAR, B. D. PETERSON, H. VAN PRAAG
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Abstract: The adult mammalian brain continuously generates new neurons in the hippocampus. The integration of newborn neurons into the existing hippocampal neuronal network is considered physiologically important for learning and memory. Enhancement of neurogenesis has been suggested to improve cognition. A simple intervention like exercise increases activity-dependent synaptic plasticity and memory function, and correlates with enhanced adult hippocampal neurogenesis. We previously described that newborn hippocampal neurons are sequentially innervated by structures important for memory function forming a unique neuronal circuit. To understand how exercise enhances learning and memory, it is essential to determine whether physical activity can modify the unique neuronal circuits of the newborn hippocampal neurons. Using a combination of retrovirus to label dividing cells and rabies virus as a retrograde tracer we identified the monosynaptic inputs to the newborn hippocampal neurons in young male C57Bl/6 mice (5-6 weeks old) in sedentary control or exercise conditions. Stereotaxic surgeries were performed to deliver the retrovirus into the right dentate gyrus (DG) of the hippocampus, and several weeks thereafter rabies virus was injected into the same DG location. Preliminary histological analysis shows that both subcortical and cortical afferents to the DG were transsynaptically labeled, and that these projections were modified by voluntary wheel running. In particular, exercise increased cortical input to the newborn granule cells, which may contribute to improved pattern separation and memory storage.

Disclosures: C. Vivar: None. **B.D. Peterson:** None. **H. van Praag:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: Wellcome Trust grant WT093072MA

Title: The influence of acetylcholine within the neurogenic niche of the spinal cord

Authors: *S. A. DEUCHARS, L. F. CORNS, L. ATKINSON, J. DANIEL, I. J. EDWARDS, J. DEUCHARS
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Abstract: The area surrounding the central canal of the spinal cord is a highly plastic area that is a postnatal neurogenic niche. Within this region are ependymal cells which may be the source of new astrocytes and oligodendrocytes following injury (Barnabe-Heider et al. (2010), Cell Stem Cell 7: 470-482) and cerebrospinal contacting cells (CSFcCs), the function of which is poorly understood. Acetylcholine in other CNS neurogenic regions has profound effects on proliferation and differentiation (Berg et al. (2013), Development 140: 2548-61); these are mediated through specific nicotinic receptors. To test whether acetylcholine may have similar effects in the spinal cord, we examined the effects of application of cholinergic drugs in acute spinal cord slices and in spinal cord slice cultures. Spinal cord slices were obtained from Urethane (2g/kg i.p.) anaesthetised Wistar rats (9-12 days old) that were transcardially perfused with sucrose aCSF and either maintained for acute whole cell patch clamp electrophysiology or prepared for organotypic culture (Hilton et al. (2006), J. Neurochem 98:690-9). All CSFcCs (n = 82) and ependymal cells (n = 36) responded to focally applied acetylcholine with a robust depolarisation; the responses in CSFcCs (15.7 ± 0.8 mV) were significantly larger than those elicited in ependymal cells (3.0 ± 0.3 mV); lack of effect of tetrodotoxin indicated that the response was direct. The non-α7-containing nicotinic cholinergic receptors (α7*nAChR) antagonist dihydro-β-erythroidinne decreased the cholinergic response in both ependymal cells (3.7 ± 0.7 mV to 1.9 ± 0.4 mV; n = 9) and CSFcCs (16.1 ± 1.8 mV to 3.0 ± 0.5 mV; n = 20, while the α7*nAChR modulator PNU 120596 significantly potentiated the remaining depolarisation in ependymal cells (1.5 ± 0.2 mV to 7.3 ± 1.9 mV; n = 4; $p < 0.05$) and all CSFcCs (3.6 ± 0.7 mV to 27.6 ± 4.0 mV; n = 10; $p < 0.001$); this revealed that both α7*nAChRs and non-α7*nAChRs were mediating the cholinergic responses. Application of α7*nAChR modulators in spinal cord cultures induced proliferation, revealed by increased EdU uptake, in ependymal cells but not CSFcCs. Our study provides evidence that acetylcholine may play a role in modulating the activity of cells within the neurogenic niche of the spinal cord.

Disclosures: S.A. Deuchars: None. **L.F. Corns:** None. **L. Atkinson:** None. **J. Daniel:** None. **I.J. Edwards:** None. **J. Deuchars:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: BBSRC Grant BB/K003534/1

Title: Environmental enrichment effects on adult hippocampal neurogenesis in mice: Dorsal vs. ventral dentate gyrus

Authors: *F. GUALTIERI[1], T. BOSWELL[2], T. V. SMULDERS[1]
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Abstract: The hippocampus is a well-defined anatomical structure belonging to the brain's limbic system that may not act as a unitary structure, with the dorsal (Septal pole) and ventral (Temporal pole) portions of it being involved in different roles. Functionally, it has always been associated with long-term memory and spatial navigation, but it also modulates corticosteroid feedback through the type I & II glucocorticoid receptors and one of its sub regions, the dentate gyrus (DG), is a source for neurogenesis. Our aim is to compare the difference in neurogenesis within dorsal and ventral DG in both standard condition and in an environmental enrichment paradigm to identify which subdivision is more sensitive. In our experiment we used 96 eleven-week-old CD-1 female mice, running four replicate experiments in each of which 24 animals were randomly divided into 4 ENRICHED and 4 CONTROL cages. Enrichment consisted of: i) running wheels, ii) increased space for social activity and iii) murine urinary proteins (MUPs) present in dirty bedding obtained from C57BL6 male mice. The control condition consisted of the standard group housing with minimal enrichment accordingly to UK 3Rs guidelines. Each replicate was conducted for 8 days and their brains were collected as follows: on third were post fixed in 4% PFA-PBS and used for morphological analyses, two thirds were processed for DG dissection. Subsequently, each dissected DG was divided in ventral and dorsal parts and rapidly frozen in dry ice for molecular biology analyses. Immunohistochemistry for Doublecortin (DCX) was carried out in 40 μm slices using the ABC indirect technique with 3,3'-Diaminobenzidine as the chromogen. Images were taken with a 20X objective on a Leica DM-LB microscope and processed with ImageJ to quantify changes in DCX positive surface area within the DG. Molecular biology analysis (quantitative PCR and protein quantification) was carried out on the dissected dorsal and ventral DG of each animal to quantify neurogenesis markers such as DCX. This study helps to clarify the functional differentiation between dorsal and ventral hippocampus and their respective sensitivity to environmental enrichment. Furthermore, using real-time PCR or ELISA assay to measure gene/protein expression allows for a quicker way to quantify hippocampal neurogenesis. This could be used to monitor the welfare condition (e.g. in terms of environmental enrichment in the home cages) of laboratory rodents, as well as in animal models aiming to investigate pathologies in which neurogenesis plays a crucial role (e.g. depression, Alzheimer's disease, etc.).

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: COST action BM1001

MESTD RS Project No. 41005

Title: The role of tenascin C in neuronal plasticity and adult neurogenesis in the hippocampus induced by enriched environment

Authors: *V. STAMENKOVIC[1], S. STAMENKOVIC[2], T. JAWORSKI[3], M. GAWLAK[3], I. JAKOVCEVSKI[4], G. M. WILCZYNSKI[3], L. KACZMAREK[3], M. SCHACHNER[4], L. RADENOVIC[2], P. R. ANDJUS[2]
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Abstract: The extracellular matrix (ECM) glycoprotein tenascin-C (TnC) plays an important role during cell proliferation, migration, neurite outgrowth and guidance early in embrional and postnatal development when it is abundantly expressed in neuronal and non-neuronal tissue. Its expression is downregulated in adult CNS with the exception of areas of plasticity and active neurogenesis. Since it is known that exposure of animals to enriched environment (EE) leads to a variety of molecular, cellular and behavioral changes, this study was designed to examine the role of TnC in: 1) structural plasticity of the hippocampus after EE by following the redistribution of perineuronal nets (PNNs) - dynamic structures involved in regulation of neuronal plasticity by immunostaining with Wisteria floribunda agglutinin (WFA), and 2) adult neurogenesis in the subgranular zone of the dentate gyrus by following markers of cell proliferation (BrdU, Ki67 and Dcx). In addition, we investigated the involvement of two ECM degrading enzymes, matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9), in these prossesess by gel and *in situ* zymography. Wild-type (TnC+/+) and TnC deficient mice (TnC-/-) were housed in standard conditions (SC) and EE for 4 and 8 weeks starting from postnatal day 21. Quantitative analysis of WFA signal intensity revealed that TnC +/+ mice reared in SC have higher PNN intensity as compared to TnC -/- mice. On the other hand, we found significant reduction of WFA signal in TnC +/+ mice reared in EE, indicating strong degradation of PNNs

and enhanced structural plasticity induced by EE, while in TnC -/- mice the effect was opposite with slight increase in PNN intensity after EE, thus implying that TnC is necessary for PNN decomposition. Furthermore, we found a significant rise in the number of BrdU, Ki67 and Dcx stained cells in TnC +/+ after EE that also occurred in TnC -/- mice, however, with higher values in both SC and EE as compared to respective groups in TnC +/+, suggesting an inhibitory effect of TnC in adult neurogenesis. Concerning MMP activity, gel zymography revealed increased MMP-9 activity after 4 weeks of EE in the hippocampus of both genotypes, while MMP-2 activity remained unchanged. However, *in situ* zymography showed significantly reduced activity of both MMPs after 8 weeks of EE in TnC +/+ and generally weak activity in TnC-/- animals. These results together emphasize a significant role of TnC in neuronal plasticity and adult neurogenesis, and also imply that interaction between TnC and MMP-9 might be critical in these processes.

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Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: NSF Cooperative Agreement Award EPS-1003907

Title: Silver nanoparticles interfere with differentiation of adult neural stem cells in culture

Authors: ***R. J. COOPER**, N. SPITZER
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Abstract: Silver nanoparticles (SNPs) have unique chemical and physical properties due to their small size and high surface area. They are widely used for their antimicrobial properties in commercial and consumer products, leading to exposure by inhalation or ingestion. At the cellular level, SNPs bypass membranes, disrupt cytoskeletal dynamics, induce oxidative stress, damage DNA, and cause apoptosis or necrosis. Recent studies indicate that SNPs breach the blood-brain barrier and accumulate in the brain. Notably, most work on SNPs examines their effects at concentrations exceeding the levels found in physiological systems, and their effects on

endogenous adult neural stem cells (NSCs) in the brain are not known. NSCs reside primarily within the subventricular zone (SVZ) of the lateral ventricles and in the hippocampus. In a healthy adult brain, these NSCs proliferate, migrate, and differentiate to integrate into existing circuits. The differentiation process results in development of mature cellular properties typical of neurons, astrocytes or oligodendroyctes *in vivo* and *in vitro*. This process of neurogenesis is a normal brain function; it is involved in learning and memory and has been implicated in endogenous repair systems. To investigate the effects of SNPs on the cellular differentiation programs of NSCs, we used an accessible model system of cultured NSCs isolated from the SVZ of young adult rats. These progenitor cells are maintained as neurospheres, and then induced to differentiate by appropriate media conditions. We observed a significant increase in the formation of f-actin inclusions when differentiating NSCs were exposed to sub-lethal levels of SNPs. Chemical enhancement of the SNPs to allow their visualization confirmed that SNPs do not co-localize with the actin inclusions. This implies that the inclusions, which are similar to those observed in response to an actin-stabilizing toxin, are formed due to an SNP-induced disruption of cytoskeletal actin dynamics, instead of being associated with sequestered SNPs. In order to further investigate cytoskeletal effects of sub-lethal SNP exposure, we used B35 neuroblastoma cells, a model for neurite extension, and time-lapse microscopy. We found that exposure to SNPs resulted in a significant decrease in maximum neurite extension. As neurite extension and dynamics are a vital part of NSC differentiation, disruption of cytoskeletal function could lead to a corresponding dysfunction of adult neurogenesis. Therefore, exposure to SNPs released from a wide variety of products may produce a deviation from healthy brain function, and a decreased capacity for learning, memory and endogenous repair.

Disclosures: **R.J. Cooper:** None. **N. Spitzer:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

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Department of Neurosurgery of Basel University Hospital

Title: Doublecortin in the cerebrospinal fluid after hypoxic-ischemic brain injury in the rat neonate is a biomarker of neurogenesis

Authors: C. BREGERE[1], U. FISCH[1], S. LIEB[1], L. CHICHA[1], P. BUSTOS[1], F. GOEPFERT[2], T. KREMER[2], *R. GUZMAN[1]
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Abstract: Study's objectives There is an unmet clinical need to monitor endogenous neurogenesis *in vivo* in developmental brain disorders. Biomarkers of neurogenesis may prove valuable for diagnostic, prognostic and therapeutic purposes. Doublecortin (DCX) is considered as a marker for neurogenesis, because it is highly expressed in migrating neuroblasts. Using a rat model of neonatal hypoxia-ischemia (HI), the study's objectives were to (i) quantify DCX in the cerebrospinal fluid (CSF) of neonates prior to and after an HI brain injury, and to (ii) examine whether DCX measured in the CSF reflects neurogenesis in the brain after HI. **Methods** Right-sided HI was elicited at postnatal day (P) 7 in Sprague-Dawley rats via ligation of the right common carotid artery and 40 minutes exposure to 8% O_2. Control animals received a sham surgery without hypoxic exposure at P7. CSF was collected before (P5) and after (P10) surgery, and DCX was quantified using a specific and highly sensitive immunoassay. BrdU (100 mg/kg) was injected intraperitoneally from P7 to P9. Animals were sacrificed at P10 and their brains were processed for immunohistochemical analysis. **Results** In sham-treated neonates, the concentration of DCX in the CSF (DCX-CSF) decreased by 85% between P5 and P10 (n=11, $p<0.0001$, paired t-test). In contrast, DCX-CSF increased by 123% in HI-injured animals (n=11) during the same time interval. Interaction between postnatal day and treatment was significant (two-way ANOVA $p=0.0197$). In the HI group, a positive correlation between DCX-CSF and stroke severity was observed. DCX immunointensity was increased in the ipsilateral subventricular zone (SVZ) and dentate gyrus from HI-injured animals in comparison to sham animals. The number of BrdU-positive cells was higher in the right SVZ from HI animals versus sham animals ($p<0.01$). **Conclusion** The decline in DCX-CSF in sham neonates between P5 and P10 is in accordance with the well-documented postnatal downregulation of DCX in the brain, and confirms that DCX is a highly developmentally regulated protein. The results after HI suggest that DCX-CSF reflects the neurogenic and proliferative responses in the brain 3 days after the insult. Overall, DCX in the CSF appears to be a valid *in vivo* biomarker of neurogenesis in the rat model of neonatal HI.

Disclosures: C. Bregere: None. **U. Fisch:** None. **S. Lieb:** None. **L. Chicha:** None. **P. Bustos:** None. **F. Goepfert:** None. **T. Kremer:** None. **R. Guzman:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Medical Scientist Training Program

Title: *In utero* lead (Pb) exposure and neuron-specific DNA methylation changes in mice

Authors: *Z. FAROOQUI[1], K. M. BAKULSKI[2], C. FAULK[1], A. BARKS[3], D. C. DOLINOY[1]
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Abstract: Abstract: Recent human post-mortem evidence indicates that frontal cortex neuronal cells have distinct DNA methylation signatures compared to non-neurons. In addition, the methylation landscape of neuronal cells varies depending on neuronal activity, based on *in vivo* research in adult mice. This evidence suggests that the methylome may be plastic, and particularly sensitive to environmental conditions such as lead (Pb) exposure. Studies in rodents and monkeys exposed to Pb in the first few weeks and first year of life, respectively, suggest that gene expression in the adult brain is associated with early life exposures. Epigenetic epidemiology research in the brain usually examines whole tissue samples comprised of mixed cell types, but it is now clear that epigenetic analyses that target specific cell types may be more informative, particularly since neurons are known to represent approximately only 10% of cells in CNS tissue. Thus, we have conducted a novel study of neuron-specific epigenetic signatures associated with Pb exposure in an isogenic mouse model (wildtype (a/a) mice of the viable yellow agouti strain). Utilizing post-mortem frontal cortex tissues from 10-month old mice exposed perinatally to Pb and controls, we optimized a Fluorescence Assisted Cell Sorting (FACS) assay to separate neuronal nuclei from non-neuronal nuclei in 3 pooled control samples (total n=9 mice) and 6 pooled Pb exposed samples (total n=19 mice). The exposure groups consisted of offspring exposed via the maternal drinking water to 0 ppm, 2.1 ppm, or 32 ppm of Pb two weeks before mating, throughout gestation, and three weeks after birth. Using NimbleGen Promoter Tiling Arrays, we probed DNA methylation levels in the neuron-specific

cell population at a genome-wide level. Using the bioinformatics bumphunting method and a family-wise error rate cutoff of 0.3, we report 3 novel exposure-dependent differentially methylated regions associated with the following genes: *Hnrnpc*, *Skint5*, and *Hnmt*. The role of *Hnmt* (histamine N-methyltransferase) is of particular interest, as it is associated with regulation of neurotransmitter levels. In environmental epigenetic studies of humans, target tissues of interest, such as the brain, are not available until post-mortem. Thus, future studies in the mouse will include an evaluation of brain-specific epigenetic targets in biologically available specimens such as blood in order to identify biomarkers of exposure and neurodegenerative disease risk.

Disclosures: Z. Farooqui: None. **K.M. Bakulski:** None. **C. Faulk:** None. **A. Barks:** None. **D.C. Dolinoy:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Program#/Poster#: 395.20/A50

Topic: A.03. Postnatal Neurogenesis

Title: Effect of housing environment on adult neurogenesis in turtles (Chrysemys picta)

Authors: *A. S. POWERS[1], B. HANUSCH[1], A. AYUNRU[1], K. HANINGTON[1], J. GOMEREZ[2], F. KERIN[1], E. LEWIS[1]
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Abstract: Adult neurogenesis has been shown to occur throughout the telencephalon in turtles (Perez-Canellas et al., 1997), but the effect of environmental variables on the amount of neurogenesis has not been extensively studied. An enriched living environment and exercise are two variables that have been shown to increase adult neurogenesis in mammals (e.g., Kempermann et al., 1997; Brown et al., 2003). We studied adult painted turtles, Chrysemys picta, housed in different conditions to determine whether there would be an effect on neurogenesis. The experiment had 3 groups of turtles: one housed in an enriched environment, including deep water, group housing, and plastic plants and a hollow log, one (called the exercise control group) given group housing and deep water but no plants or log, and one in which the turtles were housed in individual tanks and shallow water. To detect neurogenesis the turtles were administered 9 injections of BrdU (50 mg/kg) 3 times a week for three weeks at the start of the experiment. Turtles from each environment were euthanized at 1 day, 21 days and 42 days

after the last injection. The results from this experiment showed significantly more neurogenesis in the enriched environment and exercise control groups than in the individually housed turtles but no difference between the exercise control and enriched environment groups. Amount of neurogenesis did not differ by survival time. Thus exercise and social interaction by themselves are sufficient to increase adult neurogenesis in turtles, and adding objects that make the environment more stimulating does not increase the number of new neurons further. The results demonstrate that environmental factors influence neurogenesis in a reptile similar to the ancestors of mammals and suggest that the mechanisms that affect adult neurogenesis have been similar in evolution since before the appearance of mammals.

Disclosures: **A.S. Powers:** None. **B. Hanusch:** None. **A. Ayunru:** None. **K. Hanington:** None. **F. Kerin:** None. **E. Lewis:** None. **J. Gomerez:** None.

Poster

395. Postnatal Neurogenesis: Environmental and Pharmacological Regulation

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Topic: A.03. Postnatal Neurogenesis

Support: AD Williams Award 646743

NIEHS Grant ES023060

Title: Abnormal cerebellar synaptic organization induced by postnatal secondhand smoke exposure

Authors: ***P. P. MULDOON**, J. STAFFLINGER, A. OTTENS
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Abstract: In the last decade, the incidence of secondhand cigarette smoke exposure has not declined for children in the U.S. as reported by the CDC. Evidence continues to mount that environmental tobacco smoke exposure (ETS) increases behavioral and cognitive problems and a risk for mental disorders in children, independent of maternal smoking during pregnancy. This represents a significant burden on society, through treatment and disciplinary costs and also through the loss of productivity. Thus, there is a need to identify the neurobiological relationship between ETS exposure and mental health disorders. The human deficits correlated with ETS exposure suggests cerebellar involvement, which is now regarded for its regulatory function in

executive control tasks. We hypothesize that late-forming and plastic cerebellar circuitry involved in regulating fronto-cortical domains is susceptible to postnatal ETS exposure with functional consequences for executive control tasks. To assess this hypothesis we exposed Sprague-Dawley male rat pups to daily ETS (0 or 100 ug/m3 total suspended particulate) from postnatal day 8 (PD8) through PD22. At PD24 behavioral testing was performed to quantify activity and attentional control in a novel environment. We assessed organizational perturbation within lateral cerebellar circuitry induced with ETS exposure using fluorescence microscopy. We found postnatal ETS exposure induced deficits in attention and inhibitory avoidance. Furthermore, ETS exposure was shown to decrease excitatory vesicular accumulation and synapses at the granular layer (GL). However, synaptic staining revealed an increase in inhibitory synapses and vesicular accretion in the molecular layer (ML). These results suggest there is less mossy fiber input into the GL and in turn less excitatory transmission into the ML. Simultaneously there is an increase in inhibitory synapses which correlates with an increase in GABA production within the ML. Together, these findings reveal the reduced GL mossy fiber input capacity along with an increased threshold for parallel fiber excitation in order to regulate Purkinje cell tonic inhibition, resulting in dysfunctional feedback in refining executive functions. Our data point to the significant effects of ETS inhalation during postnatal critical period initial formation of higher-order circuit, impacting cerebellar organization at both the synaptic and neurotransmitter levels. Future studies will assess the effect of ETS exposure within frontal cortical domains and the underlying biochemical networks involved in this process to help us to further understand the neurodevelopmental impacts of ETS exposure.

Disclosures: P.P. Muldoon: None. **J. Stafflinger:** None. **A. Ottens:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

Support: T32 Institutional Grant

NIH Grant

Title: Alterations in tryptophan metabolism following maternal intrauterine inflammation

Authors: *M. A. WILLIAMS, L. WOICHIECH, Z. ZHANG, K. RANGARAMANUJAM, S. KANNAN
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Abstract: Tryptophan is an essential amino acid, and required for synthesis of serotonin (5HT), but upregulation of an alternate pathway towards kynurenine production reduces the amount available. The kynurenine pathway in itself is neurotoxic, with metabolites such as quinolonic acid associated with oxidative stress and excitotoxicity. Serotonin is an important neuromodulator required for brain wiring. In this study, we evaluated if intrauterine inflammation activated the kynurenine pathway of tryptophan metabolism in fetal brains and placentas, which leads to injury and 5HT depletion. Pregnant rabbits were administered E.coli endotoxin along the uterus on G28 (term=G31). A control group received no intervention. Placenta and fetal brains were harvested after 24 hours (G29), and at postnatal day 1. To investigate the tryptophan pathway we measured the mRNA levels of major enzymes that are involved in the tryptophan pathways, including IDO, KAT and KMO, as well as cytokine IFN-γ. We found that *in utero* endotoxin exposure increased the expression levels of IDO by 20-fold at G29, and ~10 fold at PND 1. The enzyme KAT2 had 2 fold change at G29, but a decrease at PND 1. KMO has had a (~7 fold-change), accompanied with an increase of IFN-γ (~50 fold-change) at G29. The expression levels of IDO (~ 10 fold-change) and IFN-γ (~12 fold-change) are still significantly higher in endotoxin animals than controls at PND1; however, the expression levels of KAT (~2 fold-change) and KMO (~2 fold-change) were significantly decreased at PND1. These results indicate that the activation of tryptophan pathway might be different at G29 and PND1; therefore, these data not only validate the feasibility of the maternal KMO inhibitor treatment, but also provide the basis for the IDO inhibitor treatment at PND1. We have concurrently measured all tryptophan and kynurenine metabolites in the placenta and fetal brain using HPLC techniques. These results are consistent in showing that tryptophan is metabolized to a greater extent along the kynurenine pathway in animals exposed to endotoxin compared to controls. Meanwhile, *in utero* endotoxin exposure slightly increased kynurenine, but significantly increased kynurenic acid level. This indicates that *in utero* endotoxin exposure increased tryptophan metabolism in the placenta along the kynurenine pathway in endotoxin group. In conclusion, maternal intrauterine inflammation results in increased activation of the kynurenine pathway in the fetal and newborn brain. This pathway may be a potent therapeutic target for suppression of toxic metabolites and for restoring serotonin levels that is crucial for the normal development of the somatosensory cortex.

Disclosures: M.A. Williams: None. **L. Woichiech:** None. **Z. Zhang:** None. **K. Rangaramanujam:** None. **S. Kannan:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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CMA BIO BIO ECM-12

Title: Adult guinea pig ventricular neurogenesis and precursor cell migration

Authors: ***N. A. JARA**[1], M. CIFUENTES[2], F. MARTÍNEZ[1], K. SALAZAR[1], F. NUALART*[1]
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Abstract: Introduction In the mouse brain, neuroblasts generated in the subventricular zone (SVZ), migrate to the olfactory bulb (OB) through a pathway called the rostral migratory stream (RMS). Although the RMS is not present in the human brain, a migratory pathway organized around a ventricular cavity (called extension of the lateral ventricles, eLV) that reaches the OB has been reported. A similar eLV structure is found in the adult guinea pig brain; however, neurogenesis and precursor cell migration has not been described in this species. Therefore, we have analyzed the neurogenic activity and precursor cell migration in the SVZ and eLV of the adult guinea pig brain. **Materials and Methods** We analyzed the SVZ, eLV and OB in 1, 6 and 12 month-old guinea pig brains. We performed bromodeoxyuridine (BrdU) labeling to analyze proliferation. Immunohistochemical analysis, confocal spectral microscopy and transmission electron microscopy were used to study the cytoarchitecture of the guinea pig SVZ and eLV. **Results** We identified neuroblasts (A cells, βIII tubulin +), precursors cells (B and C cells, BrdU +), and ependymal cells (E cells, vimentin +) in the SVZ and eLV. The eLV was lined by ependymal cells and was surrounded by migrating neuroblasts. The average number of neuroblasts per section increased progressively from the SVZ to the BO, reaching 2559 ± 164.5 neuroblasts in the OB. Ultra-structural analysis confirmed our results; the ependymal cells have cilia and microvilli on their apical surface, and neuroblasts and astrocytes were densely packed under the ependymal wall. After 24 h, BrdU labeling revealed that BrdU + cells were mainly located in the SVZ, with few BrdU + cells in the eLV and OB. In addition, the average number of BrdU + cells decreased in the SVZ and increased in the OB 1, 5 and 10 days after BrdU labeling, indicating that neuroblasts migrate from the SVZ to the OB. Finally, analysis of neurogenesis in 1, 6 and 12 month-old guinea pigs revealed that the eLV structure was preserved

in older animals; however, the average number of neuroblasts and BrdU + cells in the SVZ and eLV decreased progressively in older animals. **Conclusions** In the adult guinea pig brain, the eLV connects the LV with the OB in a fashion similar to that described in the human brain. A stream of migrating neuroblasts is organized around the eVL migration path that has no neurogenic potential. In older guinea pigs, neurogenesis decreases in a similar way to that described in other species. Therefore, we propose that the guinea pig brain may be used as a new neurogenic model with closest similarity to that observed in humans.

Disclosures: N.A. Jara: None. **M. Cifuentes:** None. **F. Martínez:** None. **K. Salazar:** None. **F. Nualart*:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

Support: NSERC Discovery Grant 402633

Ontario Graduate Scholarship

Title: Hippocampal neurogenesis in short- and long-lived rodents

Authors: D. E. PERAGINE[1], Y. YOUSUF[1], *M. M. HOLMES, PhD[2]
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Abstract: Species exhibiting remarkable adaptations, such as extended health and longevity, may reveal insights into physiology and pathogenesis that are not readily apparent in traditional animal models. Naked mole-rats (NMRs; Heterocephalus glaber) are the longest-living rodents known, with a maximum life span of 30 years- 10 times that of similarly sized laboratory mice. In addition to resistance to cancer, NMRs show attenuated declines in physical and cognitive function with advancing age. Although work with traditional short-lived laboratory rodents has established a link between age-related declines in neuronal turnover and cognition, longer-lived mammalian species exhibit low levels of neurogenic activity throughout life, suggesting that stable low rates of neurogenesis may help preserve cognitive function. To determine whether altered profiles of neurogenesis might be associated with longevity in NMRs, we compared cell proliferation and neurogenesis in NMRs and mice. At chronological and developmental stage-

matched time points (birth, infancy, weaning, sexual maturity, adulthood), brains were collected and immunohistochemically processed for Ki67 and Doublecortin (DCX), markers for cell proliferation and immature neurons, respectively. Preliminary data show comparatively low levels of hippocampal Ki67 and doublecortin (DCX) expression in NMRs relative to mice throughout early postnatal life and middle adulthood. Further, although mice exhibit striking declines in proliferative activity with increasing age, relatively stable rates are observed in NMRs. Future investigations will clarify other processes that preserve cognitive function in this species (e.g., resistance to pro-apoptic stimuli), and further develop the NMR as a model for adult neuroplasticity and successful cognitive aging.

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Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

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SNF 31-122589

Intramural Funds Univ. Zurich

Title: Adult neurogenesis in the olfactory bulb and hippocampus of the homing pigeon: A stereological lifetime study

Authors: V. MESKENAITE[1], *H.-P. LIPP[2,3]
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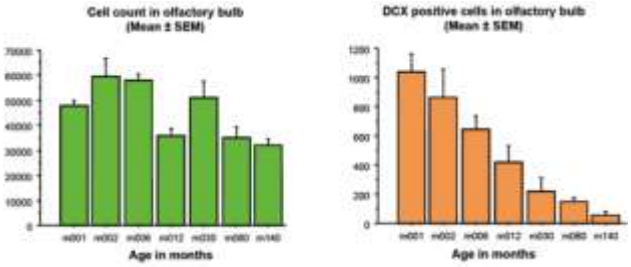
Abstract: Homing pigeons permit life time studies of brain structures because the age of the animals is routinely documented. This permits purchasing older birds without the need for long periods of housing. We have studied a sample of pigeons spanning the entire lifetime including development and ageing. Age levels included 1 month (n=6), 2 months (n=5), 6 months (n=5), 12 months (n=6), 24 months (n=4), 72-84 months (n=6) and 144-168 months (n=3). One hemisphere was used for assessing Giemsa-stained neuron numbers in the olfactory bulb (periglomerula and granule cell layer), and in the triangular area of the hippocampus, while the

other hemisphere was used for staining and quantification of neuronal proliferation by means of PCNA ((proliferating cell nuclear antigen)), and markers for young neurons such as doublecortin (DCX) and PSA-NCAM (polysialylated-neural cell adhesion molecule). In the *olfactory bulb*, *we observed a strong decrease in postnatal neurogenesis* during the first year of life. Levels of adult neurogenesis decreased further to 5-10% of the levels observed in the youngest pigeons., the number of olfactory neurons remaining fairly constant across age levels. Conversely, *in the hippocampus the number of mature neurons nearly doubled in old birds*, possibly due to increased proliferation. There was an overall reduction in the number of newly generated (DCX-positive) neurons, which possibly became integrated as adult cells. The observed decline in the olfactory bulb might be related to the *increasing attachment to their home loft*, while the changes in hippocampal neurons might reflect *increased navigational experience*.



Disclosures: **V. Meskenaite:** None. **H. Lipp:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Program#/Poster#: 396.04/A56

Topic: A.03. Postnatal Neurogenesis

Title: Protracted postnatal development of neuronal circuits in the hippocampus of naked mole rats

Authors: ***E. KEIMPEMA**[1,2], O. K. PENZ[1], J. FUZIK[2], R. ROMANOV[1], J. LARSON[3], T. J. PARK[3], T. HARKANY[1,2]
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Abstract: Naked mole rats are the longest-living rodents. Whether their longevity associates with altered postnatal neuronal turnover, diversification and network wiring remains unknown.

By using high-resolution and quantitative neuroanatomy, we show that neuronal immaturity, defined by molecular marks and the organization of synaptic connectivity and dendritic morphology of CA1-CA3 pyramidal cells and dentate granule cells, persists in the postnatal naked mole rat hippocampus until at least 4 years of age. Dendritogenesis, including dendritic spine formation, was particularly delayed. Likewise, we observed intense neurogenesis in the dentate subgranular zone with extensive migration of the resultant progeny, exceeding that in other rodents. Dentate neurogenesis ceased by 10 years of age and was associated with significant cell redistribution, neuronal apoptosis and gliosis in naked mole rats of advanced age. Correlated patch-clamp electrophysiology measurements in CA1 pyramidal neurons revealed protracted maturation of the hippocampal network, defined as the gradual acquiring of the ability to fire action potentials (APs) and by biophysical AP parameters in naked mole rats. In contrast, dentate granule cells exhibited relatively limited postnatal functional diversity, which we attributed to the rapid turnover and continuous network integration of this cell population. Overall, our data demonstrate that naked mole rats retain a heightened level of neuronal plasticity throughout a prolonged postnatal period. Moreover, our data identify the naked mole rat as a novel model for the analysis of the temporal and cellular control of neuronal network formation.

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Poster

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Topic: A.03. Postnatal Neurogenesis

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Title: Multicolor *in vivo* single cell tracking reveals the dynamic behavior of olfactory adult-born neurons during the pre-integration phase

Authors: Y. LIANG[1], K. RIECKEN[2], A. MASLYUKOV[1], D. GOMEZ-NICOLA[3], Y. KOVALCHUK[1], H. PERRY[3], B. FEHSE[2], *O. GARASCHUK[1]
[1]Univ. of Tübingen, Tübingen, Germany; [2]Res. Dept. Cell and Gene Therapy, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Ctr. for Biol. Sci., Univ. of Southampton, Southampton, United Kingdom

Abstract: The olfactory bulb (OB) is one of the brain areas that receive continuous adult-born neural progenitors (NPs) generated in the subventricular zone and the rostral migratory stream (RMS) throughout life, but the rules governing migration and integration of NPs as well as their migration patterns remain unknown. This is partly due to the difficulty of tracking single cells *in vivo* over long period of time. To monitor the migration of adult-born NPs over time, we established a two-photon-based optical imaging approach for precise identification of cell positions in the OB of live mice. This approach, which we called optical brain positioning system (oBPS), combines repetitive blood vessel imaging and red-green-blue (RGB) cell marking. Blood vessel imaging provides a consistent reference for the position of cells. RGB marking of adult-born NPs is achieved by simultaneous injection of three individual retroviral vectors encoding mCherry, Venus, and mCerulean fluorescent proteins in the RMS. RGB marking identified a group of cells expressing differential combinations of the three fluorescent proteins, assigning them a unique color hue and facilitating single-cell analysis. With this oBPS system, we were able to follow the fate of individual adult-born NPs from day-post injection (DPI) 6 onwards until DPI 60. We found that (i) adult-born cells first migrate vividly in the glomerular layer (up to 150 μm per half day) but virtually stop moving by DPI 30; (ii) the fate of cells during this pre-integration phase follows three main pathways: around one third of them settle down by DPI 30 making up the final population of integrated adult-born neurons, one third die before DPI 30, and the remaining cells likely migrate out of the field-of-view. Once in the glomerular layer, cells mostly migrate laterally, i.e. parallel to the surface of the OB. Thus, our study is the first to monitor the life cycle of adult-born NPs during the entire pre-integration phase. It reveals the surprisingly vivid exploratory behavior of adult-born NPs reflected by their long-range lateral migration in the glomerular layer of the OB. In addition, oBPS proves to be an effective tool for *in vivo* cell tracking and could also be applied to other parts of the brain, or the other tissues in live animals.

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Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

Support: Program Revenue

Title: Nestin expression and the proliferative potential of tanycytes and ependymal cells lining the walls of the 3rd ventricle in the adult rat brain

Authors: *R. E. KALIL[1], I. ZUTSHI[2], C. HASKEN[3], M. HENDRICKSON[1]
[1]Univ. of Wisconsin-Madison, MADISON, WI; [2]Univ. of California San Diego, San Diego, CA; [3]DePauw Univ., Greencastle, IN

Abstract: There is disagreement regarding the rate of cell proliferation in the ependymal cell layer lining the walls of the 3rd ventricle, and the potential of these cells to generate new neurons under normal conditions in the adult mammalian brain. To investigate the proliferative and neurogenic potential of ependymal cells and tanycytes in the walls of the 3rd ventricle, we conducted a comprehensive distribution mapping of the types and location of cells expressing the neural progenitor cell marker nestin along the entire rostral-caudal extent of the 3rd ventricle. We also determined whether ependymal cells and tanycytes in the adult rat brain proliferate under normal conditions or following an injury to the visual cortex. The distribution of proliferating cells was monitored by the incorporation of the thymidine analogue bromodeoxyuridine (BrdU) following seven daily injections of BrdU (150mg/kg) at 24 hour intervals or by the expression of Ki-67, and was compared to the distribution of cells expressing nestin. Abundant nestin was present in tanycyte cell bodies and processes and also was seen in isolated ependymal cells and in patches of ependymal cells in the walls of the 3rd ventricle. We observed very limited cell proliferation by ependymal cells or tanycytes as determined by BrdU incorporation. Typically fewer than 20 BrdU+ cells were seen throughout the rostral-caudal extent of the 3rd ventricle in each brain, and none of these cells expressed nestin. By contrast, more than 200 Ki-67+ cells were observed throughout the rostral-caudal extent of the 3rd ventricle in each brain and 13% of these cells co-expressed nestin. Most of these cells were tanycytes, but some appeared to be ependymal cells. After an injury of visual cortex, we saw no increase in cell proliferation as measured by BrdU incorporation in the walls of the 3rd ventricle. Thus, unlike the close association of nestin expression and BrdU+ cells in neurogenic regions of the brain, such as the subventricular and subgranular zones, as well as in neural progenitor cells in culture, none of the nestin-positive cells in the walls of the 3rd ventricle had incorporated BrdU following seven daily injections of BrdU. It is not surprising that the results with Ki-67 expression are different. While Ki-67 expression demonstrates that a cell has divided, it does not shed light on when this event occurred. In summary, the present results indicate that cell division in the walls of the 3rd ventricle is tightly controlled, but is not completely absent. Moreover, very few cells that have divided express nestin; indicating that the production of new neurons by cells located in the walls of the 3rd ventricle is not likely to be significant.

Disclosures: R.E. Kalil: None. **M. Hendrickson:** None. **I. Zutshi:** None. **C. Hasken:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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NS12969

Shire Pharmaceutical C.

Title: Serotonergic (5-HT) stimulation of enteric neurogenesis: identification of target neuronal phenotypes and 5-HT4 mediation

Authors: *M. D. GERSHON, A. CHALAZONITIS, M. LIU, Z. LI
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Abstract: The enteric nervous system (ENS) develops from neural crest-derived precursor cells (ENCDC). These ENCDC give rise to neurons that are born in a phenotype-related sequence. Because serotonergic neurons are among the first to terminally differentiate, they coexist with still-dividing ENCDC. As a result, 5-HT from enteric serotonergic neurons can influence the differentiation of those neurons that follow the withdrawal of serotonergic neurons from the cell cycle. Indeed, the total number of enteric neurons and particularly dopaminergic, GABAergic, and CGRP-expressing neurons, all of which are late-born, are deficient in transgenic (TPH2KO) mice, which cannot synthesize neuronal 5-HT and in mice that carry a gain-of-function mutation in SERT (G56A), which clears 5-HT from its receptors too rapidly. 5-HT4 agonists, including prucalopride, were found to stimulate enteric neurogenesis in wild-type adult mice but not in those lacking 5-HT4 receptors. We thus tested the hypothesis that 5-HT-promoted enteric neurogenesis is 5-HT4-mediated. ENCDC were isolated from E15 fetal mouse gut with antibodies to p75NTR and cultured in serum-free media. Neurons were identified immunocytochemically with antibodies to Hu proteins. 5-HT and the selective 5-HT4 agonists, prucalopride (2.5 μM) and BIMU-8 (2.5 μM), enhanced the development/survival of total enteric neurons to 135 ± 4 and 133 ± 2 % control; $p < 0.01$); however, prucalopride/BIMU-8 exerted significantly greater ($p < 0.01$) effects on development/survival of late-born neurons, expressing tyrosine hydroxylase (178 ± 17; 179 ± 12 % control; $p < 0.01$) or GABA (160 ± 3; 189 ± 19 % control; $p < 0.01$). These effects were not observed in early-born (calretinin) neurons. The 5-HT4 antagonist, GR113808 (1.0 μM), abolished the enhancement of neurogenesis in response to 5-HT, prucalopride, or BIMU-8. These observations suggest that 5-HT4 stimulation is sufficient to

account for the ability of 5-HT to promote enteric neurogenesis and that late-born enteric neurons are selectively 5-HT4-sensitive; however, further experiments are needed to determine whether other subtypes of 5-HT receptor also contribute to 5-HT-driven enteric neurogenesis.

Disclosures: **M.D. Gershon:** 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.; Shire Pharmaceutical Co.. **A. Chalazonitis:** None. **M. Liu:** None. **Z. Li:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

Support: NIMH Grant 5T32MH020030-15

Title: Alternative splicing and DNA methylation in the developing human brain

Authors: ***P. T. MANSER**[1], M. REIMERS[2]
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Abstract: Recent studies of brain development have detected global changes in gene expression, alternative splicing, and DNA methylation (Kang et al, Nature 2011; Lister et al, Science 2013). An unresolved question is to what extent these coincident genomic and epigenomic changes are actually related. Cancer studies have established DNA methylation in promoter regions as a mechanism for controlling gene expression, but few genome-wide studies have found many such effects in normal tissues. Recent studies have implicated gene body CpG methylation in and around exons as a mechanism for promoting exon inclusion (Maunakea et al, Cell Research 2013). Motivated by these findings, we employ whole-genome expression data from developing brains from the BrainSpan Project to test for correlation between changes in alternative splicing and corresponding nearby changes in DNA methylation. We develop a set of general statistical methods using canonical correlation analysis on summary measures obtained from principal component scores to test for overall association of exon inclusion and DNA methylation. We perform further analysis using obtained canonical covariates to test if observed associations correspond to differences between brain regions or developmental changes over time, or a combination of the two, and test for the plausibility of cis-acting, specific associations using a

permutation test. These methods adapt to the often sparse and irregular coverage of the Illumina HumanMethylation450 BeadChip used in the BrainSpan data. Only a quarter of hg19 exons have a CpG probe within 250 bp, and only 16% of genes with 4 or more exons have a probe within 250 bp of more than half of their exons. Nevertheless, preliminary results show an enrichment of loci-specific associations between splicing and methylation for genes involved in axon guidance, post-synaptic density, and other genes known to have multiple isoforms. Additional studies using emerging technologies assaying DNA methylation at a denser sampling of CpG sites will help further elucidate DNA methylation's role in regulating alternative splicing over brain development. Our methods are readily adapted to other microarrays, sequencing assays, and epigenetic marks.

Disclosures: P.T. Manser: None. **M. Reimers:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

Title: Immunohistochemical detection of cyclin E in postmitotic neurons of the mouse adult hippocampal dentate gyrus

Authors: *Y. IKEDA[1], M.-A. IKEDA[2]
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Abstract: Cyclin E is a member of the G1 cyclins and is essential for the G1/S transition of the cell cycle in proliferating cells. However, it has been reported recently that cyclin E plays a role in synapse function and memory formation in the adult mouse central nervous system, indicating a cell-cycle independent role for cyclin E. We have previously shown that cyclin E is expressed not only by proliferating neurons but also by postmitotic neurons in various brain regions including the cerebral cortex and the cerebellum from embryonic development through adulthood. In the present study, we studied using immunohistochemistry the detailed localization of cyclin E in the adult mouse hippocampus, where new neurons are generated from precursor cells in the subgranular zone (SGZ) of the dentate gyrus. Cyclin E expression was limited to PCNA-negative postmitotic cells located in the SGZ. Cyclin E immunoreactivity was localized to the vertical process of morphologically radial glia-like cells, and some of the cyclin E-positive

cells showed co-localization with neural stem cell markers including nestin and GFAP. Furthermore, cyclin E immunoreactivity was detected in the nucleus of cells, which were double-stained with various stage-specific markers of neurogenesis. The numbers of nuclear cyclin E-positive cells in the SGZ reduced and increased with age and running, respectively, indicating a role for cyclin E associated with neurogenic activity. We also found that the majority of nuclear cyclin E-positive cells were double-stained with active caspase-3, a marker of apoptotic cells. Collectively, these results indicate that cyclin E is expressed in the vertical process of neural stem cells and the nucleus of apoptotic neurons in the adult mouse hippocampal dentate gyrus, suggesting that subcellular localization may be important for the role of cyclin E in each cell type.

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Poster

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Support: JSPS DC1 fellowship

JSPS SPD fellowship

NIBB Collaborative Research Project 10-104

Grant-in-Aid for Scientific Research 23300115

Title: Analysis of mechanism underlying brain growth accompanied by neurogenesis using medaka fish (Oryzias latipes)

Authors: *Y. ISOE[1], T. OKUYAMA[2], M. HOKI[2], Y. SUEHIRO[1], G. YAMAGISHI[1], G. YAMAGISHI[1], K. NARUSE[3], M. KINOSHITA[4], Y. KAMEI[3], A. SHIMIZU[5], T. KUBO[2], H. TAKEUCHI[2]
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Abstract: In vertebrates, the basic brain neural networks are defined during embryonic development. The brain growth spurt occurs postnatally, accompanied by the rapid increases in

cell number and brain volume. It remains unknown, however, how postnatal (post-hatch) neurogenesis contributes to the organization of neural network required for social behaviors. To address this subject, I focused on medaka fish (Oryzias latipes), which is a model animal for molecular genetics and show prominent post-hatch brain growth and behavioral development. First, I analyzed proliferation zones in the young (sexually immature) medaka brain and identified two zones that had not been identified in the adult brain. Next I intended to generate transgenic medaka fish in which post-hatch neurogenesis can be genetically modified. And I generated the transgenic line (HuC:loxP-DsRed-loxP-GFP) that HuC promoter drives specifically in newborn neural progenitors in the adult brain. Further when stochastic recombination was induced by micro-injection of Cre mRNA into the Tg embryos at the 1 cell stage, it resulted that visualization of clonally-related cells in compartmented regions in the telencephalon in the adult medaka brain. Also, heat induction of transgenic embryo (HSP:Cre) led to Cre-recombination in the nervous system. Interestingly, by using this both lines (HSP:Cre and HuC:loxP-DsRed-loxP-GFP), heat induction can induce different Cre-recombination pattern depending on the developmental stages when heat induction was performed. As a result of analyzing stochastic recombinated samples, I identified some clonal units in the telencephalon of adult brain. Finally, by using an infrared laser-evoked gene operator (IR-LEGO) system, induction of heat shock in a micro area in the developing brains led to visualization of clonally-related HuC-expressing cells in the adult medaka fish.

Disclosures: **Y. Isoe:** None. **T. Okuyama:** None. **M. Hoki:** None. **Y. Suehiro:** None. **G. Yamagishi:** None. **G. Yamagishi:** None. **K. Naruse:** None. **M. Kinoshita:** None. **Y. Kamei:** None. **A. Shimizu:** None. **T. Kubo:** None. **H. Takeuchi:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

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NIH/NINDS Grant R01-NS070024

Title: Long-term hydrocephalus modifies the cytoarchitecture of the adult ventricular-subventricular zone

Authors: *O. GONZALEZ-PEREZ[1], T. CAMPOS-ORDONEZ[2], V. HERRANZ-PEREZ[3], K. CHAICHANA[4], D. ZARATE-LOPEZ[2], V. LOPEZ-VIRGEN[2], F. ADIRSCH[2], J. GUZMAN-MUNIZ[2], N. MOY-LOPEZ[2], J. M. GARCIA-VERDUGO[3], A. QUINONES-HINOJOSA[4]
[1]Psicologia/University of Colima, Colima, Mexico; [2]Neurosci. /Psicologia, Univ. of Colima, Colima, Mexico; [3]Inst. Cavanilles, Univ. of Valencia, Valencia, Spain; [4]Neurosurg., Johns Hopkins Univ., Baltimore, MD

Abstract: Background: Hydrocephalus can develop secondarily to a disturbance in production, flow and/or absorption of cerebrospinal fluid. Experimental models of hydrocephalus are few and limited, and the effects of hydrocephalus on the subventricular zone are unclear. Objective: To analyze the effects of long-term obstructive hydrocephalus on the subventricular zone. Methods: We developed a new method to induce hydrocephalus by obstructing the aqueduct of Sylvius in the mouse brain (P60), thus simulating aqueductal stenosis in humans. The degree of ventricular dilatation and cellular composition of the subventricular zone were studied by immunofluorescence and transmission electron microscopy (n = 18 mice per group). In adult patients (age > 18 years), the sizes of the subventricular zone, corpus callosum, and internal capsule were analyzed by magnetic resonance images obtained from patients with and without aqueductal stenosis (n = 25 per group). Mice with 60-day hydrocephalus had a reduced number of Ki67+ and doublecortin+ cells on immunofluorescence, as well as decreased number of neural progenitors and neuroblasts in the subventricular zone on electron microscopy analysis as compared to non-hydrocephalic mice. Remarkably, a number of extracellular matrix structures (fractones) contacting the ventricular lumen and blood vessels were also observed around the subventricular zone in mice with hydrocephalus. In humans, the widths of the subventricular zone, corpus callosum, and internal capsule in patients with aqueductal stenosis were significantly smaller than age and gender-matched patients without aqueductal stenosis. Conclusion: Supratentorial hydrocephalus reduces the proliferation rate of neural progenitors and modifies the cytoarchitecture and extracellular matrix compounds of the subventricular zone. In humans, this similar process reduces the subventricular niche as well as the width of corpus callosum and internal capsule.

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Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

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Title: Age-related changes of neurite density and fiber orientation dispersion in white matter during childhood brain maturation

Authors: *J. P. OWEN, Y.-S. CHANG, T. THIEU, N. POJMANN, P. BUKSHPUN, E. SHERR, P. MUKHERJEE
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Abstract: The human brain undergoes an extended period of postnatal development, with white matter (WM) maturation continuing through adolescence and even into adulthood. Studies using diffusion tensor imaging (DTI), a technique which provides information about tissue microstructure, have demonstrated widespread, nonlinearly increasing fractional anisotropy (FA) over age. However, DTI lacks the specificity to disentangle individual microstructural features such as myelination and axonal fiber density. Neurite orientation dispersion and density imaging (NODDI) offers advantages as a multi-compartmental biophysical model of WM microstructure (Zhang et al., 2012) that can infer the non-collinear properties of fiber orientation dispersion (OD) and neurite density (ND), corresponding to the degree of incoherence in fiber orientations and to the intracellular volume fraction, respectively. In WM, ND can be considered equivalent to axonal density. In this work, we study WM maturation in a group of 68 human subjects (ages 7-63 years, 29 female, 39 male) using both DTI and NODDI. We also quantify the test-retest reliability of FA, OD, and ND in 5 of these adult subjects who were each scanned 2-4 times. High resolution 3T structural MRI was acquired, as well as whole-brain diffusion-weighted MRI using 30 directions at b=1000 s/mm^2 and 64 directions at b=3000 s/mm^2. Postprocessing included motion and eddy current correction, brain extraction, and DTI as well as NODDI fitting to calculate maps of FA, OD and ND. Using Tract-Based Spatial Statistics (TBSS) in FSL, the FA maps from all subjects were registered to and skeletonized using FSL's MNI152-registered FMRIB FA template. Global values of FA, OD and ND were obtained for each subject by averaging over each whole-brain skeletonized WM map. Additionally, regional tract values were obtained for each subject from WM skeleton voxels using the ICBM-DTI-81 White Matter Labeled Atlas. We find that ND increases significantly and globally in WM throughout late childhood, adolescence and adulthood through middle age, while OD exhibits weaker increases globally in WM, and, in fast-maturing central white matter tracts, remains relatively constant through late childhood, adolescence and adulthood through middle age. ND and OD demonstrate comparable test-retest reliability to that of FA, with coefficients of variation of 1.8% (OD), 1.0%

(ND), and 1.1% (FA) for the global WM skeleton. We conclude that the rising FA of human white matter development from childhood to young adulthood is driven by large increases of ND, i.e., axonal density, which outweighs the smaller age-related increases of OD that would otherwise cause diminishing FA.

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Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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NYS OMH

Title: Ectopic granule cells in the dentate gyrus of normal mice: Dependence on age, septotemporal location, and survival of hilar progenitors

Authors: *K. BERMUDEZ-HERNANDEZ[1,2], J. J. LAFRANCOIS[1], J. MORETTO[1], H. E. SCHARFMAN[1,2]
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Abstract: The migration of neurons to their correct destination is essential for normal function. We and others have shown that mismigrated neurons in the dentate gyrus (DG) can develop in pathological conditions. In many rodent models of temporal lobe epilepsy (TLE) substantial numbers of mismigrated granule cells (GCs) are found in the hilus, and there is a positive correlation between seizure frequency and the numbers of these hilar “ectopic” GCs (hEGCs). Remarkably, it is not known to what extent mismigrated GCs exist normally. Furthermore, it is not clear if all hEGCs are caused by mismigration, because they may develop simply because hilar progenitors (and their progeny) survive. The goal of this study was to determine 1) whether hEGCs exist in normal mice, 2) their septotemporal distribution, and 3) if increasing survival of progenitors early in postnatal development could lead to an increase in hEGCs. To address these questions we quantified hEGCs, defined by the GC marker Prox1 and neuronal marker NeuN

using C57B6/J mice at postnatal day (P) 16 (n=4), P30 (n=5), and P60 (n=4). In addition, we increased survival of progenitors and their daughter cells by removing the proapoptotic gene BAX with a tamoxifen (TAM) injection at P2-P8 using NestinCreERT2 Baxfl/fl mice (NCBaxfl/fl). Animals were examined at P60 (TAM-treated, NCBaxfl/fl, n=7; TAM-treated, Baxfl/fl, n=7; vehicle-treated, NCBaxfl/fl, n=3; vehicle-treated, Baxfl/fl, n= 7). To quantify hEGCs along the septotemporal axis we separated one whole hippocampus from the rest of the brain and sectioned it perpendicular to the long axis (1 every 5th section). We also used conventional coronal and horizontal sections. The highest hilar Prox1 cell density was at P16, followed by P30 and then P60 (one-way ANOVA, $p< 0.0001$). Preliminary results suggest that at P16 most of the Prox1 cells in the hilus do not express NeuN but this proportion increases substantially after P30 suggesting a role of puberty (Fisher's exact test, $P<0.0001$). The density of Prox1 cells was highest in the septal hippocampus regardless of age (two-way ANOVA, $p<0.0001$). Also, increasing the survival of nestin-expressing progenitors and their daughter cells postnatally increased hilar Prox1 cell density at P60 (One-way ANOVA, $p= 0.0003$). The results suggest that 1) hEGCs exist normally and continue to develop during puberty, 2) the septal DG is the primary site of hEGCs and 3) hEGCs can form by increasing survival of hilar progenitors. We suggest that early life is a time when the DG is susceptible to hEGC formation because of a large population of hilar progenitors at that age.

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Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

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Topic: A.03. Postnatal Neurogenesis

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National Natural Science Foundation of China (31121061 and 91232723)

Title: Human and monkey striatal interneurons are derived from the medial ganglionic eminence but not from the adult subventricular zone

Authors: D. QI, *Z. LIU, C. WANG, Y. YOU, X. ZHOU, S. WEI, Z. ZHANG, W. HUANG, F. LIU, Z. YANG
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Abstract: In the adult rodent and monkey brain, newly-born neurons in the subventricular zone (SVZ) in the wall of the lateral ventricle migrate into the olfactory bulb (OB) via the rostral migratory stream (RMS). However, a recent study reported that there are constantly generating neurons in the adult human striatum from the SVZ. In contrast, by taking advantage of the continuous expression of the zinc finger transcription factor Sp8 from the neuroblast stage through differentiation into mature interneurons, we found that the adult human SVZ does not generate new interneurons for the striatum.In the adult human SVZ and RMS, very few neuroblasts were observed and most of them expressed Sp8. Neuroblasts in the adult rhesus monkey SVZ-RMS-OB pathway also expressed Sp8. In addition, we observed that Sp8 was expressed by most adult human and monkey OB interneurons. However, very few Sp8+ cells were in the adult human striatum. This suggests that neuroblasts in the adult human SVZ and RMS are destined for the OB, but not for the striatum. BrdU-labeling results also revealed few if any newly-born neurons in the adult rhesus monkey striatum. Finally, on the basis of transcription factor expression, we provide strong evidence that the vast majority of primate interneurons in the striatum are generated from the medial ganglionic eminence during embryonic developmental stages, as they are in rodents. We conclude that, although a small number of neuroblasts exist in the adult human SVZ, they do not migrate into the striatum and become mature striatal interneurons.

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Poster

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National Natural Science Foundation of China (31121061 and 91232723)

Title: Human and monkey striatal interneurons are derived from the medial ganglionic eminence but not from the adult subventricular zone

Authors: ***D. QI**[1,2], Z. LIU[1], C. WANG[1,3], Y. YOU[1], X. ZHOU[1], S. WEI[1], Z. ZHANG[1], W. HUANG[1], F. LIU[1], Z. YANG[1]
[1]Inst. of Brain Sci. and State Key Lab. of Med. Neurobio., Fudan Univ., Shanghai, China; [2]Dept. of Neurobio., Xuzhou Med. Col., Xuzhou, China; [3]Dept. of Neurol., Affiliated Hosp. of Hebei Univ. of Engin., Handan, China

Abstract: In the adult rodent and monkey brain, newly-born neurons in the subventricular zone (SVZ) in the wall of the lateral ventricle migrate into the olfactory bulb (OB) via the rostral migratory stream (RMS). However, a recent study reported that there are constantly generating neurons in the adult human striatum from the SVZ. In contrast, by taking advantage of the continuous expression of the zinc finger transcription factor Sp8 from the neuroblast stage through differentiation into mature interneurons, we found that the adult human SVZ does not generate new interneurons for the striatum.In the adult human SVZ and RMS, very few neuroblasts were observed and most of them expressed Sp8. Neuroblasts in the adult rhesus monkey SVZ-RMS-OB pathway also expressed Sp8. In addition, we observed that Sp8 was expressed by most adult human and monkey OB interneurons. However, very few Sp8+ cells were in the adult human striatum. This suggests that neuroblasts in the adult human SVZ and RMS are destined for the OB, but not for the striatum. BrdU-labeling results also revealed few if any newly-born neurons in the adult rhesus monkey striatum. Finally, on the basis of transcription factor expression, we provide strong evidence that the vast majority of primate interneurons in the striatum are generated from the medial ganglionic eminence during embryonic developmental stages, as they are in rodents. We conclude that, although a small number of neuroblasts exist in the adult human SVZ, they do not migrate into the striatum and become mature striatal interneurons.

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Poster

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Title: The fine structure of adult neural stem cells in the hippocampal neurogenic niche

Authors: *J. MOSS[1], E. BUSHONG[2], M. H. ELLISMAN[2], N. TONI[1]
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Abstract: New neurons are born into the dentate gyrus throughout adult life. They develop from a pool of self-renewing radial glia-like (RGL) stem cells, which divide and differentiate into mature granule cells that are capable of functionally integrating into the hippocampal circuit. The RGL stem cells that give rise to new neurons have a very curious morphology. From their subgranular cell bodies, they extend a substantial primary process radially through the densely-packed granule cell layer to the molecular layer. This process then splits into secondary processes that branch extensively, forming a dense plexus of fine glia-like processes. This complex structure raises a couple of questions: 1) Does their structure enable the stem cell to interact with other components of the neurogenic niche? 2) What signals could they be receiving, and for what purpose? To begin answering these questions, we first examined the fine structure of RGL stem cell processes using light and electron microscopy. Nestin-expressing RGL stem cells were labelled in a Nestin-GFP transgenic mouse, using either an immunoperoxidase or immunogold protocol, before imaging. Analyses showed that the fine processes of the stem cell possessed mitochondria-containing varicosities at regular intervals, which gave rise to further processes that wrapped local synapses in a glia-like fashion. However, due to the large number of stem cell processes, very few astrocytic processes could infiltrate the same region. Determining the neurochemical nature of the stem cell-wrapped synapses is currently underway. Processes also wrapped around nearby blood vessels, with a pronounced thickening and more cytosolic mitochondria where they met. When the primary process of the stem cell grew into a region containing a blood vessel, its regularly branching tree of processes became warped, with some secondary processes targetting the blood vessel and others maintaining a regular structure separately. Analysing the fine structure of RGL stem cell processes has revealed a close relationship with both local synapses and blood vessels within the neurogenic niche. This suggests that RGL stem cells could use their fine processes to assess their local environment and determine if new neurons were needed, or might survive, in their vicinity.

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Poster

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Support: NSERC

Title: Survival and maturation of the developmentally-born cell population in the rat dentate gyrus

Authors: *S. P. CAHILL, R. Q. YU, J. S. SNYDER
Dept. of Psychology, Univ. of British of Columbia, Vancouver, BC, Canada

Abstract: The discovery that neurons are added to the adult brain of nearly all mammals examined, including humans, has had profound effects on our understanding of the potential for plasticity in the brain. In the dentate gyrus, adult-born neurons have enhanced plasticity and contribute to the mnemonic and emotional functions of the hippocampus. Furthermore, the stages of adult neurogenesis and the factors that regulate neuron addition in adulthood have been relatively well-characterized. In contrast, less is known about how developmentally born cells integrate and survive over time and how these processes are regulated by experience. Since the dentate gyrus is comprised of large numbers of adult-born and developmentally-born cells, identifying the properties of these two populations is essential for understanding how the dentate gyrus, as whole, contributes to behavior. To address this question we used BrdU to label dentate gyrus neurons born on postnatal day 6 in rat pups, the peak of dentate gyrus development. We quantified BrdU+ cells in 2 and 6 month-old rats and found 30% fewer BrdU+ neurons at the 6 month time point, indicating substantial loss of mature, developmentally-born cells throughout adulthood. This contrasts with adult-born cells, which show stable survival after reaching maturity. Despite the significant death of developmentally-born cells, our preliminary results suggest that this cell death can be offset by exercise between 2 and 6 months of age. Ongoing experiments will additionally examine the survival and maturation of developmentally-born cells at earlier timepoints, similar to the work that has been done in characterizing adult neurogenesis. Collectively, our findings indicate that the survival and turnover of developmentally-born cells greatly influences the composition of the adult dentate gyrus. The sensitivity of these cells to experience also suggests they may make novel contributions to dentate gyrus plasticity throughout adulthood. These findings are relevant for understanding the role of the dentate gyrus in learning and memory as well as conditions such as aging and disease where neurons are lost.

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Poster

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William Owens Medical Research Foundation Grant

Title: Calorie restriction prevents age-related decreases in neurogenesis and preserves the neural stem cell niche

Authors: ***D. M. APPLE**, R. S. FONSECA, C. ZHU, S. MAHESULA, E. KOKOVAY
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Abstract: The aging brain faces many challenges- memory and cognitive deficits, structural changes, and decreased plasticity. Aging is a major risk factor for increased susceptibility to damage from brain insults like stroke, inflammation, and disease. As the brain ages, it loses the capacity to make new neurons, which decreases its ability to respond to damage. Protecting the brain's potential to produce new neurons from neural stem cells is paramount for maintaining healthy brain aging. Calorie restriction (CR) can improve physiological markers of health during aging, including extending lifespan and protecting against age-related damage to the brain. The largest source of neural stem cells in the adult brain is harbored in the subventricular zone, but it is unknown if this neural stem cell population is altered by CR. We sought to determine the effect of CR on neurogenesis and neural stem cell survival in the subventricular zone of young and aged mice. Consistent with published reports, aged mice fed standard control chow showed fewer subventricular zone derived neurons in the olfactory bulb, indicating that aging impairs neural stem cell function. CR preserved neural stem cell function and resulted in a significant increase in neurogenesis in aged mice compared with ad libitum- fed controls. Furthermore, we observed a marked increase in cellular senescence in the subventricular zone in aged mice on control diet compared to young mice. This was significantly reduced in aged CR mice, which displayed rare senescent cells with expression levels similar to young mice. Confocal imaging and fluorescent staining of subventricular zone wholemounts revealed an increase in both the total number and reactivity of microglia in the aged control mouse, suggesting increased

inflammation in the neural stem cell niche during aging. Remarkably, these age-related inflammatory markers were not observed in the CR aged mice, which appeared no different from young control and young CR mice included in the study. These initial experiments have revealed a protective role for CR in the aging subventricular zone, and are an important first step in understanding how CR may be an effective therapeutic intervention for the aging or damaged brain.

Disclosures: D.M. Apple: None. **R.S. Fonseca:** None. **C. Zhu:** None. **S. Mahesula:** None. **E. Kokovay:** None.

Poster

396. Postnatal Neurogenesis: Temporal and Spatial Patterns

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 396.19/B3

Topic: A.03. Postnatal Neurogenesis

Support: NIH-NCI P30 CA54174

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Owen's Medical Research Foundation Grant

Nathan Shock Center of Excellence in Basic Biology of Aging Pilot Grant

Title: Inflammation in the aging neural stem cell niche

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Abstract: The subventricular zone (SVZ) is the largest reservoir of neural stem cells (NSCs) in the adult brain. Neurogenesis is reduced during aging and this decline in NSC function is thought to contribute to reduced brain function. Here we propose immune activation as a contributor to age-related declines in NSC function. We observed a sharp decrease in neurogenesis in mice between the ages of six and twelve months. Neuroblast number was also reduced with age. Microglia are the prominent immune cells in the brain. Microglia were observed throughout the SVZ niche and were observed at the level of the ependymal compartment and the vasculature plexus. At the ependymal compartment, microglia processes can be observed intercalated between ependymal cells and close to pinwheel structures. Microglial activation results in the

release of proinflammatory cytokines. Interestingly, the number of microglia cells in the SVZ was observed to increase during aging. Furthermore, both morphological changes and increased CD68 expression were observed during aging, indicative of microglia becoming more activated. In conditioned media (CM) experiments, a higher amount of cleaved Caspase-3 staining was observed in NSC cultures using microglia CM, compared to controls indicative of higher levels of apoptosis. In addition, β-tubulin staining revealed a reduction in neurogenesis when microglia CM was used compared to controls. Our results suggest that during aging there is increased inflammation in the SVZ which may be an important contributor to declines in neural stem cell function.

Disclosures: R. Solano Fonseca: None. **R. Raghunathan:** None. **A. Dugan:** None. **E. Kokovay:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.01/B4

Topic: A.04. Stem Cells

Support: NIH1R15 ES019298-01A1

Title: Rapid generation of sub-type specific neurons and neural network from human pluripotent stem cells derived neurosphere

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Abstract: Rapid and efficient generation of sub-type specific neurons and neural networks from neurosphere provide the platform for disease modeling and cell therapy. In neurotransplantation, neurospheres are commonly used as neuroprogenitors but neurosphere derived subtype specific neurons or their region specificity is not yet well established. Neurosphere derived neuronal culture usually stays as clump which makes them very difficult to study. Here, we describe a detailed protocol for direct differentiation of different layers of cortical, pyramidal, GABAergic, glutamatergic, cholinergic and dopaminergic neurons from human embryonic and induced pluripotent stem cells (h/iPSCs) derived neurosphere. In this protocol, we provide a total 27 days of neuronal differentiation timeline including generation of fully functional neurons which is

faster than traditional protocol takes about 80-days. The neurons derived from this protocol have fully functional synapses which spontaneously active to fire action potentials and form neural network *in vitro*. Two critical additional steps are novel in this protocol, first step is to initiate the neuronal differentiation with knockout serum replacement medium (KSRM) and incubate the cells at 10% CO2. We found 10% CO2 culture condition increased 2-folds neuroprogenitors gene expression compared to 5% CO2. Second, we introduced an AdSTEP mechanical procedure to generate smaller fragments that facilitates the robust and homogenous neural population which always been challenging for neurosphere based neuronal culture. Furthermore, we also provide side by side comparison of our neuroshere with standard neuroectoderm based culture and found neuroprogenitors from neurosphere stable overtime and continuously can give rise large percentage of neurons compared to neruoectoderm based neuronal culture. Therefore, neurosphere based neuronal culture is valuable tool to study molecular mechanisms of neurogenesis, neural development and cellular therapeutics for a variety of neurological diseases.

Disclosures: A.N. Begum: None. **Y. Hong:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.02/B5

Topic: A.04. Stem Cells

Title: 17q21.31/WNT3-WNT9b CNVs accelerates dopaminergic neuron differentiation from hPSCs

Authors: *C.-T. LEE, A. A. KINDBERG, R. M. BENDRIEM, L. T. WORDEN, M. P. WILLIAMS, T. DRGON, B. S. MALLON, B. K. HARVEY, C. T. RICHIE, R. S. HAMILTON, G. R. UHL, W. J. FREED
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Abstract: Prolonged culture of hPSCs can lead to chromosomal abnormalities as well as more subtle genetic alterations, any of which can affect the properties of hPSC lines. We examined associations between copy number variations (CNVs) on chromosome 17 and hPSC mesodiencephalic dopaminergic (mDA) differentiation. Five karyotypically-normal hPSC lines (BG01, 02, 03, ES02, 04) and one trisomy 17 line (BG01V2) were examined. All displayed similar survival rates and normal morphology. BG01V2 and BG03 showed enhanced rates of proliferation, and lost pluripotency more rapidly than other cell lines following withdrawal of

basic fibroblast growth factor (bFGF). BG01V2 and BG03 also displayed rapid mDA differentiation, with earlier generation of $LMX1A^+$ mDA progenitors and TH/ $TUJ1^+$ DA neurons. Since BG01V2, with trisomy 17, underwent rapid mDA differentiation, CNV analysis was focused on chromosome 17. A genomic duplication located at 17q21.31 was identified in BG03, encompassing the *WNT3-WNT9* gene cluster, two candidates for involvement in mDA differentiation. Additional copies of *WNT3* and *WNT9B* in both BG01V2 and BG03 were confirmed by qPCR. We also screened an additional 18 hPSC lines for extra copies of *WNT3*/*WNT9B*. Among several hPSC lines with increased levels of *WNT3* and/or *WNT9B* DNA, CT3 was examined and showed rapid differentiation similar to that of BG01V2 and BG03. WNT3 and WNT9B act *via* the β-catenin (canonical) and the Rho/JNK (non-canonical) signaling pathways, respectively. To examine the roles of *WNT3-WNT9B* CNVs in mDA differentiation, we used canonical (DKK-1) and non-canonical (SP600125) signaling inhibitors as well as lentiviral vectors to overexpress or silence *WNT3* and *WNT9B* expression. In hPSC with amplified *WNT3-WNT9B*, DKK-1 treatment or WNT3 knockdown reversed both enhanced proliferation in the undifferentiated state, and accelerated mDA differentiation, decreasing mDA markers including LMX1A, TUJ1, and TH in cells with amplified *WNT3/WNT9B*. SP600125 treatment or WNT9B knockdown reversed the accelerated loss of pluripotency in hPSCs with amplified *WNT3-WNT9B*. Conversely, in control hPSCs WNT3 overexpression in increased undifferentiated proliferation and mDA differentiation, while WNT9B overexpression accelerated departure from pluripotency. Thus amplification of *WNT3* and *WNT9B* causes multiple changes in hPSC function and mDA differentiation. Enhanced non-canonical Rho/JNK pathway signaling *via* WNT9B promotes early differentiation and departure from pluripotency, while enhanced canonical/β-catenin signaling *via* WNT3 accelerates subsequent mDA specification *via* up-regulation of LMX1A.

Disclosures: C. Lee: None. **A.A. Kindberg:** None. **R.M. Bendriem:** None. **L.T. Worden:** None. **M.P. Williams:** None. **T. Drgon:** None. **B.S. Mallon:** None. **B.K. Harvey:** None. **C.T. Richie:** None. **R.S. Hamilton:** None. **G.R. Uhl:** None. **W.J. Freed:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.03/B6

Topic: A.04. Stem Cells

Title: Differentiation of astrocyte with human embryonic stem cells

Authors: ***B.-S. HAN**[1], C.-O. LEE[2], S.-Y. KIM[2], S.-C. LEE[2]
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Abstract: Neurons, astrocytes, and oligodendrocytes-the three major cell types that comprise the central nervous system-are generated from common multipotent stem cells. Many reports have shown that astrocytes can be generated from various tissue sources including human pluripotent stem cells (PSC). In this presentation, we describe a chemically defined medium culture system for rapidly generating astrocytes from neural precursor cells derived from human embryonic stem cells. We found that neural and astrocyte precursor stage had the advantage for making pure astrocytes. Gene expression patterns were investigated in astrocytes generated by our method. We have tried to investigate the role of some genes during astrocyte differentiation.

Disclosures: B. Han: None. **C. Lee:** None. **S. Lee:** None. **S. Kim:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.04/B7

Topic: A.04. Stem Cells

Title: Using human embryonic stem cells to understand *in vitro* cortical excitatory neurogenesis and lineage

Authors: ***S. KU**, J. CLOSE, B. GREGOR, J. GRIMLEY, A. JAYABALU, A.-R. KROSTAG, B. LEVI, R. MARTINEZ, R. MAY, V. MENON, H. MULHOLLAND, A. NELSON, K. NGO, N. SHAPOVALOVA, M. SMITH, C. THOMPSON, E. THOMSEN, A. WALL, Y. WANG
Allen Inst. For Brain Sci., Seattle, WA

Abstract: *In vivo* human brain development remains one of the greatest challenges of scientific understanding, and while previous efforts at the Allen Institute for Brain Science have focused on the adult human brain, the *In vitro* Human Cell Types program is currently taking an alternative approach by studying *in vitro* differentiation and development using human pluripotent stem cells. Specifically, using a modified version of a previously developed method for neural induction via dual-SMAD inhibition, we are differentiating embryonic stem cells (ESCs) to forebrain cortical progenitors followed by maturation into cortical excitatory neurons. At pre-determined time points, cells are collected and analyzed by flow cytometry,

immunocytochemistry, Fluidigm qRT-PCR, and population and single-cell RNAseq to interrogate the gene expression changes that occur and/or changes that may be causal. Additionally, we employ a cell line engineering platform that allows for the efficient generation of targeted, genome-modified ESC lines of interest, including reporter and knockout lines. The use of these reporter lines then allows us to isolate specific cell populations of interest to further investigate their gene expression profiles as well as developmental potential/lineage. These approaches, together with various computational methods for analyzing these data, will allow us to generate hypothetical "maps" of neuronal differentiation and cell lineage relationships. Subsequently, iterative testing of these hypothetical maps using the same analytical tools we have developed as well as engineered reporter or knockout ESC lines will allow us to refine our lineage map, ultimately helping to further our understanding of cortical development.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: NIH Grant NS075893

PD Council Grant

Title: Authentic midbrain dopamine differentiation of pluripotent stem cells requires the coordinated regulation of En2, Foxa2 and Lmx1a expression

Authors: ***J. CAI**, E. W. KOSTUK, L. M. IACOVITTI
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Abstract: Midbrain dopamine (mDA) neurons derived from human pluripotent stem cells (hES or hiPSC) are a promising cell source to be used in cell replacement therapy of Parkinson Disease (PD) or as a PD cell model. In recent years, small molecule inhibitors and agonists have been added to the differentiation protocols to produce mDA neurons, including the BMP

inhibitor dorsomorphin (DM), TGF β inhibitor SB431542 (SB) and SHH agonist purmorphamine (Pur). Previously, we developed a protocol which mimics mDA differentiation *in vivo* by combining these molecules with the growth factor FGF8. Here, using a careful time course study, we examine the effects of individual or combined molecules on mDA differentiation in monolayer hES cell cultures. We find that all four substances in our cocktail are necessary to produce a true mDA phenotype in stem cells. DM alone promotes pan-neurogenesis including GABAergic and Glutamergic neurons. Neither SB nor Pur alone efficiently drive neurogenesis or an mDA phenotype in cells. Only the combined treatment of all 4 reagents yielded cells labeled by mDA markers Lmx1a, Foxa2 and TH. Importantly, these cells also expressed high levels of En2 both at the neural progenitor stage and the mDA neuron stage. This is the first demonstration that in addition to Lmx1a and Foxa2, En2, must be expressed in cells with an authentic mDA phenotype, a proposition supported by our mouse developmental studies. Finally, in our previous studies, the transcription factor SMAD-interacting protein (SIP1) was identified as a key player regulating mDA differentiation following induction by SMAD inhibitors DM/SB in our cocktail. Importantly, expression levels for all 3 mDA-specifying genes (Lmx1a, Foxa2, En2) were dramatically reduced in SIP1 knockdown experiments, indicating their coordinated regulation by SIP1. Ongoing studies are aimed at understanding the pathways regulating an authentic mDA differentiated phenotype in stem cells.

Disclosures: **J. Cai:** None. **E.W. Kostuk:** None. **L.M. Iacovitti:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

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CTSI-CN

The Scott-Gentle Foundation/NARSAD

Kavli Institute for Neuroscience at Yale

Title: Involvement of heterogeneous activation of Heat Shock Factor 1 in the formation of focal cortical dysplasia elicited by prenatal environmental challenges

Authors: *S. ISHII[1], M. RAJENDRAPRASAD[1], A. SON[1], Y. MOROZOV[2], A. NAKAI[3], V. MEZGER[4,5], P. RAKIC[2], M. TORII[1,2,6], K. HASHIMOTO-TORII[1,2]
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Abstract: Prenatal environmental challenges, such as maternal stroke and drug use affect fetal cortical development, thereby increase the risk of intellectual disability, epilepsy and so on. Heat Shock Factor 1 (HSF1) - Heat Shock Protein (HSP) signaling (HSF1-HSP signaling) is required to protect the embryonic cortex from exposure to a variety of environmental challenges, thereby reduces the risk of the mental disorders (Hashimoto-Torii K. et al, Neuron, 2014). In contrast to general assumption that HSF1-HSP signaling is equipped equally by most types of the cells for their protection, here, we show that HSF1-HSP signaling is activated at various levels among the cells in the fetal cortices exposed to the environmental challenges and that this variable activation may be a cause of cortical dysplasia. First we tested HSF1-HSP signaling activation in human iPSCs-derived neural stem/progenitor cells (NSPCs) under exposure to ethanol, hydrogen peroxide, and methyl mercury by quantifying the number of HSP70 mRNA using single molecule Fluorescence *In situ* Hybridization (smFISH). In contrast to the consistent expression of housekeeping genes including GAPDH, HSP70 expression shows significant variability among NSPCs in response to these environmental challenges. This result suggests that HSF1-HSP signaling is activated at different level in individual cells by a variety of environmental challenges. By generating *in vivo* mouse fluorescence reporter system for the HSF1-HSP signaling, we also confirmed the heterogeneity of the activation in fetal cortices exposed to environmental challenges. We next examined potential effects of the bursty activation of the signaling that is induced in a part of cells exposed to the challenges on cortical development. By introducing constitutively active form of HSF1 (caHSF1) into normal embryonic cortex, we found that the pyramidal neurons electroporated with the caHSF1 impaired the radial migration. Importantly, we also found that the impaired neuronal migration caused by exposure to challenges is mitigated by reducing such bursty activation of the HSF1-HSP signaling. Altogether, these results demonstrate that a part of cortical cells may activate protective signaling at excess level in response to prenatal environmental challenges, and that this excess activation adversely affects the cortical development.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: Norwegian Research Council

Helse Sør Est

Title: Expression of 5-HT receptors in human blastocysts and ESCs

Authors: *A. SAMARA[1,2], S. STRÖM[1,2], L. ANTONSSON[3], R. LAMPELA[3], J.-T. KIEHN[4], J. C. GLOVER[1,2,5]
[1]Univ. of Oslo, Oslo, Norway; [2]Norwegian Ctr. for Stem Cell Res., Oslo, Norway; [3]Karolinska Intitutet, Stockhom, Sweden; [4]Univ. of Lübeck, Lübeck, Germany; [5]Sars Intl. Ctr. for Marine Mol. Biol., Bergen, Norway

Abstract: Serotonin (5-hydroxytryptamine, 5HT) is a major neuromodulatory neurotransmitter and hormone in nearly all animal species. In mammals, 5HT has been implicated as a regulator of neurodevelopmental processes and alterations of the fetal 5HT supply can have adverse effects on neural circuit formation [1]. The physiological effects of serotonin range from regulation of subcortical sensory and motor systems, to regulation of higher cognitive functions. Deregulation of serotonin is implicated in a myriad of neuropathologies, including pain syndromes, motor, sleep, eating and mood disorders. The effects of serotonin are exerted via 7 subfamilies of 5HT receptors (5HTRs) [2]. The 5HTRs comprise 6 families of GPCRs (5HT-1, 2, 4, 5, 6, and 7) and one ligand-gated ion channel (5HT3). Little is known, however, about the effects of 5HT in human embryonic stem cells (hESC). To address this question, after confirming the expression of 5HT receptors in day 5 human blastocysts, we tested 15 hESC lines, namely HS181, 207, 306, 360, 364, 380, 401, 415, 420, 429, 799, 980, 999, 1001 and H9, for expression of all 5HTRs and 4 of them were tested for changes in cell signaling and gene expression after exposure to 5HT. METHODS Cell culture: In brief, hES cell lines were either cultured on human foreskin fibroblasts in 20% KO-SR in KO-DMEM supplemented with bFGF, or on laminin coated dishes in NutriStem hESC XF medium; 100 μM 5HT was added to hESC culture medium for 24h. Real time RT-PCR was performed to assess the expression of 5HTRs. RESULTS Transcripts of all 5HTRs subtypes are present in hESCs and were all upregulated after culture medium supplementation with 5HT for 24hrs. Moreoever, exposure to 5HT resulted in upregulation of gene expression of stem cell markers, proneural genes, 5HT specification-

related transcription factors and serotonergic pathway genes. DISCUSSION Currently, very few 5HTR ligands are subtype-selective and development of novel, specific 5HT antagonists is needed. Pharmaceutical R&D has mainly been compromised by a lack of experimental platforms. Characterization of 5HTR expression profiles is important for developing appropriate tools for 5HTR subtype-specific assays. References [1] Bonnin et al. (2007) Nature Neuroscience 10:588-597. [2] Raymond et al. (2001) Pharmacology and Therapeutics 92:179-212.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

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Program#/Poster#: 397.08/B11

Topic: A.04. Stem Cells

Title: Development and characterization of scalable human induced pluripotent stem cell-derived midbrain dopaminergic neurons for drug discovery and disease modeling

Authors: ***L. CHASE**, C. MCMAHON, J. MA, J. GRINAGER, N. MEYER, C. CHAVEZ, B. MELINE, J. LIU, C. CARLSON, K. MANGAN, W. WANG, B. SWANSON
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Abstract: Since the discovery of human induced pluripotent stem cells (iPSCs), much excitement and interest has been created around this technology as a platform for generating pluripotent stem cell lines from a range of specific genetic backgrounds, both normal and diseased. Using an optimized episomally-derived human iPSC platform, we have developed highly consistent and scalable differentiation protocols for making various types of human neurons. As described here, based upon previously published work (Kriks et al. 2011), we have further optimized and developed a scalable method for the generation of differentiated, cryopreserved human midbrain dopaminergic neurons (iCell DopaNeurons). This protocol provides a consistent platform to study various aspects of midbrain dopaminergic neuron biology, including Parkinson's disease. Phenotypically, iCell DopaNeurons are floor plate-derived midbrain dopaminergic neurons. During differentiation, these cultures go through a highly pure midbrain progenitor phase as shown by high level expression of forkhead box A2 (FoxA2) and the LIM homeobox transcription factor 1a/b (Lmx1). Upon further differentiation,

midbrain dopaminergic neurons are generated as identified by expression of microtubule-associated protein 2 (Map2), FoxA2, Lmx1 and tyrosine hydroxylase (TH). Utilizing a genetic-based selection strategy, the differentiating dopaminergic neuron cultures are purified to >90% neurons as determined by expression of Map2 and the absence of the progenitor marker nestin. Upon thaw, iCell DopaNeurons quickly acquire a branched neuronal morphology and display midbrain dopaminergic markers. In addition, these cultures maintain a high level of dopaminergic neuron purity for extended culture post-thaw. Functionally, these cells display characteristic neuronal electrophysiological properties, including proper ion channel activity, fire both evoked and spontaneous action potentials and show excitatory post-synaptic currents. In addition, through the use of multielectrode arrays, these cells display characteristic excitatory phenotypes with responses to known pharmacological agents. Finally, as a proof-of-concept for the ability to scale out a neuronal differentiation process, episomally-derived human iPSCs from various donors were created and differentiated into a highly purified neuronal population, exemplifying the potential application of this technology to panels of donors as a means to study larger human populations.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Support: Maryland Stem Cell Research Foundation

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NIH 5P30EY001765

Knights Templar Eye Foundation

Foundation Fighting Blindness

Research to Prevent Blindness

Title: Genome-engineering human induced pluripotent stem cell derived 3D retinas to model photoreceptor development and disease

Authors: *K. WAHLIN[1], J. MARUOTTI[2], V. RANGANATHAN[2], C. KIM[2], V. SLUCH[2], S. SRIPATHI[2], C. BERLINICKE[2], D. J. ZACK[3]
[1]Johns Hopkins - Wilmer Eye Inst., BALTIMORE, MD; [2]Ophthalmology, [3]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Background and Significance. Retinal degenerative diseases, such retinitis pigmentosa (RP), cause dysfunction and death of photoreceptor (PR) cells leading to blindness. For most retinal degenerations there is no cure and significant gaps exist in our understanding of how PR loss occurs. To address this we are developing gene-modified human induced pluripotent stem cell (hiPSC) retinal cell-reporters and RP-associated hiPSCs that will help to identify pathways promoting retinal differentiation and potentially uncover pathways leading to PR survival. The feasibility of this approach is supported by recent work showing that hiPSCs can be coaxed into becoming retinal eyecup-like structures with complex laminar morphology and photoreceptor-like cells. Our approach bridges three technologies: (1) hiPSCs to generate 3D-differentiated retinas, (2) small molecule chemical screening to identify pathways that increase PR generation, and (3) genome-editing using CRISPR technology to generate genetically matched retinal reporters and disease-based mutant hiPSCs. A main goal of our work is to identify novel mechanisms for PR development that could increase the efficiency and pace of PR generation and provide insight into human ocular development. Methods. To differentiate hiPSCs toward a retinal fate we have adopted and further modified a forced aggregate approach pioneered by Nakano et al (2012) whereby stem cells are dissociated and re-aggregated to a defined size. In the presence of small molecules to mimic pathways intrinsic to retinal development and matrigel to mimic the ECM environment of the retina *in vivo*, human retinal eyecups are generated that parallel human retinal development. To engineer retinal reporters (e.g. SIX6-H2BGFP) into our cell lines we have adopted the CRISPR/Cas9 genome editing molecular scissor technology to knock-in fluorescent proteins in frame into the 3'-end of retinal gene coding sequences. The CRISPR/Cas9 system has also been used to generate CRX mutations similar to those found in human patients with Leber congenital amaurosis (LCA), and autosomal dominant RP. Results. We can now reliably generate retinal eyecups with complex laminar morphologies that develop exuberant outgrowth of both rod and cone outer segment-like structures, hallmark features of mature neural retinas. Using the above genome-editing approach we have also generated several retinal reporter cell lines, one of which is a SIX6-H2BGFP iPSC line that is a reliable marker for early retina. The eyecup protocol together with these CRISPR/Cas9 reporter lines will be used to identify and characterize pathways that accelerate retinal formation.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: AFA försäkring, Swedish Research Council (K2013-61X14603-11-5)

Deutsche Forschungsgemeinschaft (DFG) SFB TR3-05/D2, the 7FP Project Neurostemcellrepair and the Hertie Foundation

Title: Optogenetics reveal delayed afferent synaptogenesis on grafted human induced pluripotent stem cell-derived neural progenitors

Authors: *N. AVALIANI[1], A. T. SORENSEN[1], M. LEDRI[1], J. BENGZON[1], P. KOCH[2], O. BRUSTLE[2], K. DEISSEROTH[3], M. ANDERSSON[1], M. KOKAIA[1]
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Abstract: Reprogramming of somatic cells into a pluripotent stem cell state have given new opportunities for cell replacement therapy and disease modeling in a number of neurological disorders. It still remains unknown, however, to what degree the grafted human induced pluripotent stem cells (hiPSCs) differentiate into a functional neuronal phenotype and if they synaptically integrate into the host circuitry. Here we present a detailed characterization of the functional properties and synaptic integration of hiPSC-derived neurons grafted in an *in vitro* model of hyperexcitable epileptic tissue, namely organotypic hippocampal slice cultures (OHSC), and in adult rats *in vivo*. The hiPSCs were first differentiated into long term self-renewing neuroepithelial stem (lt-NES) cells, which are known to form primarily GABAergic neurons. When differentiated in the OHSCs for six weeks, lt-NES cell-derived neurons displayed neuronal properties such as TTX-sensitive sodium currents and action potentials (APs), as well as both spontaneous and induced postsynaptic currents, indicating functional afferent synaptic inputs. They had a distinct electrophysiological profile compared to host cells in the OHSCs with higher input resistance, lower resting membrane potential and APs with lower amplitude and a longer duration. To investigate the origin of synaptic afferents to the grafted lt-NES cell-derived neurons, the host neurons were transduced with Channelrhodopsin-2 (ChR2) and optogenetically activated by blue light. Simultaneous recordings of synaptic currents in grafted lt-NES cell-derived neurons using whole-cell patch-clamp technique at 6 weeks after grafting revealed limited synaptic connections from the host neurons. Longer differentiation times, up to 24 weeks

after grafting *in vivo*, revealed more mature intrinsic properties and extensive synaptic afferents from host neurons to the It-NES cell-derived neurons, suggesting that these cells require extended time for the differentiation/maturation and synaptogenesis. However, even at this later time-point, the grafted cells maintained a higher input resistance. These data indicate that grafted lt-NES cell-derived neurons receive ample afferent input from the host brain. Since the lt-NES cells used in this study show a strong propensity for GABAergic differentiation, the host-to-graft synaptic afferents may facilitate inhibitory neurotransmitter release, and normalize hyperexcitable neuronal networks in brain diseases, e.g. such as epilepsy.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.11/B14

Topic: A.04. Stem Cells

Title: Somatic cell lines with targeted eGFP insertion into mouse Tph2 locus as a model for serotonergic transdifferentiation

Authors: ***N. ALENINA**[1], V. FISHMAN[2], S. MIGLIARINI[3], B. PELOSI[3], A. RANJAN[1], O. SEROV[2], M. BADER[1], M. PASQUALETTI[3]
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Abstract: The investigation of serotonergic (5-HT) neuron activity and its relationship to disease has been limited by a lack of physiologically relevant *in vitro* cell models. Differentiation of pluripotent stem cell lines or transdifferentiation of somatic cells carrying a 5-HT neuron-specific reporter provides a platform for such studies and can serve as a model for drug discovery. In this study we aimed to generate 5-HT neurons from somatic cells directly or via formation of induced pluripotent stem (iPS) cells using a serotonergic reporter mouse line. As a source of somatic cells we took primary mouse embryonic fibroblasts (MEFs) carrying the eGFP gene targeted to the endogenous Tph2 locus (Tph2:eGFP MEFs, Migliarini et al., 2013). Using these MEFs we first generated iPS cells by transfection with a single polycistronic lentivirus carrying Oct4, Sox2, cMyc, and Klf4 (OSKM) genes driven by a doxycycline-inducible promoter. The resulting iPS cells showed ES cell-like morphology and expressed the

pluripotency-associated genes Nanog and Oct4, but not eGFP. To test the potential of Tph2:eGFP-iPS cells to produce 5-HT neurons we induced neuronal differentiation by formation of embryoid bodies following replating on PDL/Laminin substrate. First eGFP-positive (eGFP+) cells with neuronal morphology appeared 14 days after induction of differentiation. Immunohistochemical analysis revealed that all eGFP+ cells express the neuronal marker, Tuj1, and produce serotonin. We detected a high expression of 5-HT markers, such as Tph2 by qPCR in the eGFP+ cell population isolated by FACS, further confirming the 5-HT nature of eGFP-expressing cells. We next took advantage of Tph2:eGFP MEFs to monitor the transdifferentiation of somatic cells to 5-HT neurons by two different strategies: (1) direct conversion using a neuron-specific cocktail of reprogramming factors (Ascl1, Brn2, Myt1l, (ABM)) in combination with transcription factors important for serotonergic differentiation, (2) via induction of pluripotency genes in culture conditions permissive for the formation of 5-HT neurons. Induction of neuronal genes (ABM) led to the robust generation of neurons, but not of eGFP+ cells. In contrast, transfection of Tph2:eGFP MEFs with OSKM lentiviruses and cultivation in LIF-free conditions in neuronal differentiation medium led to the appearance of eGFP+ neurons after 25 days. In conclusion, we generated a useful model system to monitor 5-HT neurons in *in vitro* reprogramming experiments. To our knowledge, this is the first successful transdifferentiation of somatic cells to serotonergic phenotype. References: Migliarini et al., 2013, Mol Psychiatry 18(10):1106-18

Disclosures: **N. Alenina:** None. **V. Fishman:** None. **S. Migliarini:** None. **B. Pelosi:** None. **A. Ranjan:** None. **O. Serov:** None. **M. Bader:** None. **M. Pasqualetti:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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NSFC/RGC-Joint Research Scheme N_HKU741/11

Innovation and Technology Fund 100/10

SK Yee Medical Research Fund

Title: Directed differentiation of human induced pluripotent stem cells to sensory neurons by combined small molecule inhibitors

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Abstract: Human induced pluripotent stem cells (iPSCs) hold great promise for cell therapies and tissue engineering. iPSC-derived neural cells provide prospects for studying neurodevelopment and modeling neurological diseases. A prerequisite for these studies is a reproducible protocol that efficiently yields an abundant number of neural cell types. However, differentiation of iPSCs with extrinsic factors is a slow, step-wise process, mimicking the complex timing of human development. Progress has been made in identifying signaling pathways that direct the differentiation of iPSCs into specific lineages. Using a combination of small molecule inhibitors, we attempt to develop a new protocol to generate sensory neurons from human iPSCs. Human iPSCs were exposed to small molecule inhibitors. The iPSC-derived sensory neurons were maintained for two weeks in growth factors required for their survival. After differentiation, over 90% of the total cell population expressed the neuron-specific protein, Tuj-1 and neurofillament. Nearly 80% of the total cell population co-expressed peripherin and Brn3a, the specific markers for sensory neuron. Therefore, we demonstrated that a combination of small-molecule inhibitors of signaling pathways promotes highly efficient peripheral neural induction from human iPSCs. Our *in vitro* model of iPSC-derived sensory neuron provides a promising strategy for controlled production of sensory neurons and may serve as a useful tool for studying human neurodevelopment and modeling neurological diseases.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: NIH grant EY019052

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Title: Differentiation of retinal ganglion cells and photoreceptor precursors from mouse iPS cells carrying a Math5/Atoh7 lineage reporter

Authors: *X. YANG[1], B.-B. XIE[2], X. ZHANG[1], T. HASHIMOTO[1], A. TIEN[1], A. CHEN[1], J. GE[2]
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Abstract: The neural retina constitutes a critical part of the visual system, which provides the majority of sensory inputs in humans. Various retinal degenerative diseases can result in the permanent loss of retinal neurons, especially the light-sensing photoreceptor cells and retinal ganglion cells (RGCs), which are the projection neurons connecting the retina with the higher visual centers in the brain. In optic nerve neuropathy and glaucoma, RGCs undergo cell death leading to decline of the vision. The repair of RGC damage is particularly challenging, as both RGC specification and the subsequent axonal growth and projection require complex and precise regulation. To begin addressing the serious roadblocks in RGC production and repair using stem cell technology, we have established mouse iPS cells that are genetically marked for a transcription factor, Math5/Atoh7, which is critical for RGC fate specification. Math5-Cre knock-in mice were crossed with Rosa.YFP cre reporter mice to derive Math5-Cre(KI);Rosa.YFP MEFs. Lentiviruses expressing the Yamanaka factors under TetO control were then used to induce the MEFs into Math5-Cre; Rosa.YFP iPS cells that express pluripotent markers and are capable of generating teratomas in SCID mice. Embryoid bodies of Math5-Cre.Rosa.YFP iPSCs were formed under anterior neural induction conditions, and further differentiated under retinal neurogenic conditions. Immunocytochemistry detected neurons co-expressing various RGC markers and YFP with extensive neurites and/or axons, indicating that these neurons were derived from Math5 lineage. Consistent with previous *in vivo* cell lineage studies, Math5 YFP-expressing cells also gave rise to a subset of photoreceptor precursors. Furthermore, FACS analyses showed that inhibition of Notch signaling in the Math5 iPSC cultures enhanced YFP reporter positive cells, and increased the Brn3a+YFP+ cells from 8.1+0.8 to 12.5+0.9 %, and Crx+YFP+ population from 7.3+1.1 to 16.4+1.9 %. Together, these results indicate that the Math5 reporter iPS cells can be used to demarcate a sub-lineage derived from retinal progenitor cells, and to study the development and survival of RGCs and photoreceptors.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division (grant number CBM.THRTOX.01.10.RC.021)

Title: Activation of trans-synaptic signaling and network formation in human stem cell-derived neurons

Authors: *K. HUBBARD, P. BESKE, E. GLOTFELTY, T. HAMILTON, P. MCNUTT
USAMRICD, APG, MD

Abstract: Synaptically active neurons derived from human pluripotent stem cells hold great potential as a physiologically relevant platform for studying development, synaptogenesis and responses to neuromodulatory conditions. However, methods to reproducibly generate synaptically active populations with emergent network behaviors have not been described. We produced human stem cell-derived neurons (hSNs) using a well-described differentiation protocol and evaluated their morphological and functional characteristics. hSNs expressed and appropriately compartmentalized neurotypic markers and electrophysiological characterization demonstrated immature neuronal characteristics. Although transmission electron microscopy revealed the presence of pre- and post-synaptic architectures, evidence of synaptic activity was not observed. These data suggested that despite expressing many of the characteristics required for synaptic activity, hSNs produced using this method remained functionally immature. To address the discrepancy between apparent morphological maturity and functional immaturity, we used a developmentally inspired approach to identify cellular processes involved in neuronal specification and patterning *in vivo* in an attempt to identify compounds that had the potential to accelerate neuronal maturation *in vitro*. We determined that a temporally phased application of a subset of these compounds produced hSNs with significantly improved morphologies, intrinsic electrical characteristics and, most importantly, AMPA receptor-mediated post-synaptic currents. Additionally, we tested the ability of two commercially available human stem cell-derived neuron models to form functioning neuronal networks. Both commercial lines expressed and appropriately compartmentalized neurotypic markers and expressed intrinsic electrical properties consistent with a neuronal identity, however neither demonstrated evidence of AMPA receptor-mediated post-synaptic currents. These data suggest that hSNs produced using developmentally inspired conditions may provide an improved method to generate synaptically active, defined

populations of human neurons appropriate for the development of novel therapeutics, toxin screening and basic research. *Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government*.

Disclosures: K. Hubbard: None. **P. Beske:** None. **E. Glotfelty:** None. **T. Hamilton:** None. **P. McNutt:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Support: Health and Labour Sciences Research Grants

Research on Regulatory Science of Pharamaceuticals and Medical Devices

JSPS Grant-in-Aid for Young Scientists (B)

Title: Axonal polarity formation in human iPSCs-derived neurons

Authors: *N. KOGANEZAWA[1], Y. OHARA[1], H. YAMAZAKI[1], R. T. ROPPONGI[1], K. SATO[2], Y. SEKINO[2], T. SHIRAO[1]
[1]Gunma Univ. Grad. Sch. of Med., Gunma, Japan; [2]Natl. Inst. of Hlth. Sci., Tokyo, Japan

Abstract: Recent advances in human induced pluripotent stem cells (hiPSCs) offer new possibilities for biomedical research and clinical applications. Differentiated neurons from hiPSCs are expected to be good tools for developing new methods of treatments for various neurological diseases. However, the detail processes of neuronal development from hiPSCs have not been known. In this study we analyzed development of hiPSCs-derived neurons, particularly focusing on their early developmental stages. We cultured iCell Neuron (Cellular Dynamics International) and compared their development with that of the primary cultured neurons derived from rat hippocampus. In 2 days *in vitro* (DIV) culture of the rat neurons, we observed three different stages which were stages 1, 2 and 3 in developmental classification proposed by Dotti. Most developed stage 3 neurons had several short neurites with one long neurite, which is destined for an axon. According to the classification of Dotti, stage 3 neurons are characterized by this one long neurite. However in the DIV 2 iCell Neuron, we observed a few stage 3 neurons

and most of the neurons were in their stage 1 or 2. This suggests that the human neurons develop slower than the rat neurons morphologically. To investigate if the axonal differentiation had occurred even though iCell Neuron showed slower development, we used anti-phosphorylated neurofilament. There were quite a few neurons with phosphorylated neurofilament positive neuritis (axons) in DIV 2 iCell Neuron. This indicates that although the axonal growth speed of iCell Neuron is slower compare to that of rat neurons, the polarity of iCell Neuron was normal. We further double labeled these cells with drebrin and F-actin to see what makes this difference between iCell Neuron and rat neurons. The main structural components of filopodia and lamellipodia are F-actin, which is modulated with an actin-binding protein drebrin. We found that the localization patterns of F-actin and drebrin in growth cones of iCell Neuron were similar to those of rat neurons. Moreover to test if there are any differences between those neurons in terms of reaction to a drug, we treated both neurons with Cytochalasin D (Cyto D), an inhibitor of actin polymerization. The effects of Cyto D on both neurons were comparable. These results indicate that iCell Neuron develops comparably to the rat neurons in regard to axonal differentiation, but the growth speed of axon is slower.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Program#/Poster#: 397.16/B19

Topic: A.04. Stem Cells

Title: MicroRNA profiling during human brain development

Authors: ***M. E. JONSSON**[1], J. NELANDER[2], M. ÅKERBLOM[1], A. KIRKEBY[2], M. PARMAR[2], J. JAKOBSSON[1]
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Abstract: MicroRNAs (miRNAs) have been shown to be involved in developmental processes and specification of different cell types. We set out to identify and investigate the expression of miRNAs during the human neural development by using isolated neural progenitors from both differentiating human embryonic stem cell (hESC) cultures and human embryos. hECSs were differentiated and regionalized towards neural progenitors of ventral forebrain, ventral midbrain

and ventral hindbrain. Small RNAs were then isolated from the different neural progenitor populations, as well as from three non-neuronal cell populations (hESCs, human lung fibroblasts and mesendodermal cells). We performed global miRNA profiling using next-generation sequencing, providing us with a spatial miRNA fingerprint of ventralized neural progenitors along the anterior-posterior axis as well as potential pan-neuronal miRNAs. Although *in vitro* differentiation of hECSs can recapitulate many aspects of the developing human nervous system we next wanted to validate the expression of the miRNAs in corresponding *in vivo* progenitors. This was achieved by dissecting corresponding brain regions from human embryos at different ages during neurogenesis (5-11 weeks post conception) and performing a multiplex qRT-PCR miRNA array. By this approach, we could again look at the spatial distribution of miRNAs and thus confirm their expression in human fetal brain, but also the temporal distribution. Our data support the existence of microRNAs that are regulated by either temporal or spatial aspects of development. We are currently performing gain- and loss-of-function studies in hESCs to investigate the functional role of specific miRNAs with these properties.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

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Topic: A.04. Stem Cells

Title: The generation of subgroups of GABAergic interneurons in 3D alginate hydrogel

Authors: *J. KIM[1], S. ANDERSON[1,2]
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Abstract: Since dysfunction of GABAergic interneurons has been associated with schizophrenia and autism, the generation of distinct ventral interneuron types has important clinical implications. In rodents, the parvalbumin (PV) and somatostatin (SST) expressing subgroups account for 60% of cortical interneurons (45% PV, 15% SST). Both subgroups originate mainly in the medial ganglionic eminence (MGE) of the basal forebrain. Evidence from the Anderson lab suggests a strong bias for SST+ interneurons to originate from ventricular zone (VZ) divisions of radial glial cells (RGC), while PV+ interneurons are generated from subventricular

zone (SVZ) divisions of intermediate progenitor cells (IPCs). Embryonic stem cells have great potential in studying the fate determination of cortical interneurons because they have ability to self-renew as well as to differentiate into specific interneurons given the appropriate signals. In a previous study from the Anderson lab, 2D stem-cell cultures, FACS sorted for a fluorescent reporter of interneuron-fated precursors and transplanted into neonatal neocortex of mice, mainly produced SST interneurons, recapitulating the VZ-like microenvironment. If so, a 3D system might better recapitulate the SVZ-like microenvironment, resulting in enhanced production of PV-expressing interneurons. We chose 3D alginate hydrogel because alginate is a major component of the ECM in the brain and has the similar surface stiffness to the brain tissue. To test our hypothesis using 3D alginate hydrogel, we are growing mouse embryonic stem cells of a dual-reporter line; Nkx2.1-mCherry for MGE progenitors, and Lhx6-GFP for post-mitotic, fate committed interneurons. The line is grown in either 2D or 3D (alginate) culture, and then MGE-like progenitors or newly born interneurons isolated by FACS are assessed by marker analysis for VZ or SVZ cells and by fate analysis 30 days after transplant into neonatal neocortex. If successful, the system will be an excellent platform for further study of cortical interneuron genesis and fate determination.

Disclosures: J. Kim: None. **S. Anderson:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: Keio University Medical Science Fund

Title: Direct differentiation of human pluripotent stem cells into cerebellar Purkinje cells

Authors: *K. IMAIZUMI, T. SONE, W. AKAMATSU, H. OKANO
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Abstract: Purkinje cells (PCs) are the sole output from the cerebellar cortex and the dysfunction of PCs leads various neurological diseases including ataxia, autism and multiple sclerosis. To model cerebellar disorders *in vitro* for drug discovery, PCs derived from patients with such cerebellar disorders have been expected. Although recent reprogramming technologies facilitated making iPSCs from the somatic cell of patients, induction of PCs from human pluripotent stem

cells have not been reported. Here, we aim to establish an efficient method to generate PCs from human pluripotent stem cells. We first demonstrated the induction of neural progenitors with specific regional identities. We showed that it was possible to control the anteroposterior identities, ranging from the telencephalon to the spinal cord, and the dorsoventral identities at any given anteroposterior position by combinatory treatment of various patterning factors during neural induction. Using this system, we successfully induced neural progenitors with the characteristics of the dorsal anterior hindbrain where the cerebellum anlage arises. To accelerate the differentiation and maturation of PCs, we next overexpressed master genes of cerebellar development into the cells with characteristics of the dorsal anterior hindbrain. 15 days later of overexpression, we detected PCs markers, such as calbindin, along with increased LHX1/5 expression. These results indicate that PC-like cells might be efficiently generated from human pluripotent stem cells. Our study will elucidate not only the pathogenesis of cerebellar disorders, but also the mechanism of cerebellar development.

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Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Title: Assessing functional phenotypic complexity of stem cell-derived neuronal culture network activity in relation to brain region-specific primary cultures

Authors: *A. GRAMOWSKI, K. JÜGELT, C. EHNERT, A.-M. PIELKA, A. PODßUN, B. M. BADER, O. SCHROEDER
NeuroProof GmbH, Rostock, Germany

Abstract: The option of using human stem cells and even personalized patient cultures will bridge in-vitro assays closer to man. Primary neuronal cultures have a long tradition and are well characterized and validated. There is a plethora of literature data documenting their physiological relevance in research and drug discovery. Neuronal cell cultures derived from murine and human stem cells are in the focus of international research now. Yet, one of the most important issues is their physiological relevance. This question can not be answered in general, but a lot of empirical data contribute to a more and more comprehensive picture. We aim to understand and compare the difference of electrical functional activity patterns from primary murine neuronal cell

cultures and those cultures derived from embryonic stem cells cultivated on micro-electrode arrays (MEA). As a result of their phenotypic receptor and neuron type composition, primary neuronal cell cultures show very specific and complex activity pattern after four weeks *in vitro*. This complexity results from a high level of organization in network cultures, which is present in primary cultures but distinguishable from those derived from stem cells. Thus, we are able to compare but also classify the complexity of stem cell-derived activity patterns in comparison to the current standard of primary cells. We compared primary neuron/glia cultures from different brain tissues such as frontal cortex, hippocampus, hypothalamus, midbrain, a midbrain/frontal cortex co-culture, spinal cord with dorsal root ganglia, and murine stem cell derived neuronal networks grown on MEAs for 4 weeks *in vitro* and a randomly generated spike train pattern generated with a Poisson process. Primary cell cultures show brain region-specific activity pattern which can be clearly distinguished by pattern recognition methods. We describe and analyze these activity patterns with 204 spike train parameters computed by in-house software NPWaveX. We show that the pattern complexity from stem cell-derived cultures is sorted between those generated with a randomly generated Poisson process spike train and primary cultures. Thus, we provide a tool to optimize the culture condition towards a higher functional network complexity.

Disclosures: **A. Gramowski:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH. **K. Jügelt:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **C. Ehnert:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **A. Pielka:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **A. Podßun:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **B.M. Bader:** A. Employment/Salary (full or part-time):; NeuroProof GmbH. **O. Schroeder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Title: Neurofluor™ Cdr3: A fluorescent probe for the detection of live CNS and human pluripotent stem cell derived neural progenitor cells

Authors: *V. M. LEE, C. K. H. MAK, S. LLOYD-BURTON, A. C. EAVES, T. E. THOMAS, S. A. LOUIS
Res. & Develop., STEMCELL Technologies Inc, Vancouver, BC, Canada

Abstract: Identification and phenotypic characterization of neural cell types derived from either the central nervous system (CNS) or human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells, is heavily reliant on antibodies and mRNA probes. Although these reagents can be highly effective and specific, their protocols are labor intensive, often requiring optimization, and involve fixation and permeabilization steps that sacrifice cell samples. Simple reagents that can quickly and selectively label live neural progenitor cells (NPCs) in tissues and cell cultures are therefore highly desirable. We have developed NeuroFluor™ CDr3, a ready-to-use fluorescent membrane-permeant probe that specifically labels live NPCs. NeuroFluor™ CDr3 was developed based on CDr3, a boron-dipyrromethane (BODIPY) derivative compound that was first identified in a screen for human and rodent CNS-derived NPC probes (Yun et al. 2012; Leong et al. 2013). Here, we confirm that NeuroFluor™ CDr3 selectively labels NPCs from embryonic rodent CNS and further demonstrate its utility in the detection of hPSC-derived NPCs. NPCs were generated from hPSCs via an embryoid body (EB) protocol, using STEMdiff™ Neural Induction Medium (NIM) and AggreWell™800. In this method neural rosettes, a morphological hallmark of *in vitro* neural differentiation were efficiently generated. These rosettes contain CNS-type NPCs that express PAX6 and Nestin. NeuroFluor™ CDr3 was diluted in NIM at various concentrations, added directly to cultures containing neural rosettes, and incubated for 2 hours at 37oC. After 2 washes, NeuroFluor™ CDr3 signal was observed with a fluorescent microscope. We found that NeuroFluor™ CDr3 used at either 0.5 or 1 μM, specifically labeled cells within the neural rosettes structures but not the surrounding cells. Finally, we generated adherent monolayer NPC cultures from E18 rat cortices using the NeuroCult™ Proliferation Kit. NeuroFluor™ CDr3 was applied directly to monolayer NPC cultures in the NeuroCult™ Proliferation medium, incubated, and washed as above. All of the cells in these cultures were positive for the neural progenitor marker, Nestin and the majority (>95%) were co-labeled by NeuroFluor™ CDr3 as visualized by fluorescent microscopy. Together, these data demonstrate that NeuroFluor™ CDr3 is a useful tool for the detection of live hPSC- and rodent CNS-derived NPCs in culture. Because NeuroFluor™ CDr3 is compatible with flow cytometry protocols, it can be applied in cell sorting strategies for the isolation and enrichment of NPCs directly from live cultures and CNS tissue samples.

Disclosures: V.M. Lee: A. Employment/Salary (full or part-time):; STEMCELL Technologies. **C.K.H. Mak:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **S. Lloyd-Burton:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **A.C. Eaves:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **T.E. Thomas:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **S.A. Louis:** A. Employment/Salary (full or part-time):; STEMCELL Technologies.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: NIH 1 PO1 GM081619-01

Title: The role of miRNAs in the developmental timing of embryonic stem cell-derived retina

Authors: *A. HOSHINO, A. LA TORRE, L. E. HOOD, T. A. REH
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Abstract: Retinal degenerative diseases are a diverse group of disorders that affect millions of people worldwide and can lead to irreversible vision impairment and blindness. In many diseases, patients initially lose one type of retinal neuron (commonly the photoreceptors), followed by the loss of other cell types. Although significant progress has been made in understanding the molecular mechanisms behind retinal degeneration, current therapies do not restore vision after retinal cells are lost. Cell therapy may thus be a promising treatment option to replace lost neurons and to recover vision. While many sources of retinal cells have been suggested, their self-renewal and pluripotent properties make embryonic stem cells (ESCs) an ideal source for generating large numbers of photoreceptors through directed differentiation. We and other groups have therefore developed protocols to differentiate ESCs into retinal lineages; these protocols recapitulate key stages of retinal development, such as the formation of the optic vesicle, optic cup, and production of different retinal neurons in a stereotypic manner. Although these results are promising, it takes several weeks to months to produce photoreceptors from mouse and human ESCs, respectively; hence speeding up differentiation will make ESCs a more viable therapeutic option. Recent work has suggested that microRNAs (miRNAs) play an important role in controlling developmental timing in the mouse retina; however, the role of miRNAs during retinal differentiation of ESCs has not yet been studied. Our laboratory has identified key miRNAs (Let7, miR-9, miR-125b, termed Late Progenitor miRNAs) that increase during retinal development, and upon precocious overexpression, can accelerate the timing of mouse neurogenesis. The current project was designed to determine whether LP-miRNAs play a similar role during retinal differentiation of ESCs. Here, we characterized the expression patterns of LP-miRNAs in the retinal cells derived from mESCs using RT-qPCR and miRNA sensors. Our analysis shows that LP-miRNAs are upregulated and functional in mESCs from the earliest stages of retinal differentiation. We then manipulated miRNA expression in the mESCs and found that early changes in miRNA levels altered the timing of key retinal differentiation events.

These data suggest that miRNAs play an important role during directed retinal differentiation of mESCs, and that manipulation of miRNAs can potentially be employed to derive retinal neurons more efficiently.

Disclosures: **A. Hoshino:** None. **A. La Torre:** None. **L.E. Hood:** None. **T.A. Reh:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 397.22/B25

Topic: A.04. Stem Cells

Support: Connecticut Stem Cell Established Investigator

Connecticut Multi-investigator stem cell award

Citizens United for Research in Epilepsy

Title: Derivation, differentiation and characterization of human and mouse embryonic stem cell derived gabaergic interneuron progenitors

Authors: *N. C. ANDERSON[1], C. Y. CHEN[2], D. F. MOAKLEY[2], K. HENDERSON[2], A. PLOCIK[3], B. GRAVELEY[3], J. NAEGELE[2], L. GRABEL[2]
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Abstract: The selective loss of GABAergic inhibitory interneurons is characteristic of numerous neurodegenerative diseases. Absence of these inhibitory subtypes creates an electrical imbalance in the hippocampal and cortical neural circuits. Our long term goal is to replenish these inhibitory interneuron subtypes using an embryonic stem cell (ESC) source. During embryonic development, these inhibitory interneuron progenitors arise from a transient ventral forebrain structure known as the medial ganglionic eminence (MGE) and are characterized by the expression of Nkx2.1. We have optimized an adherent monolayer protocol for the generation of Nkx2.1+ neural progenitors from both mouse and human ESC lines using sonic hedgehog treatment. The Nkx2.1+ enriched cell population expresses elevated levels of MGE markers, including Nkx2.1 and Nkx6.2, based upon qRT-PCR analysis. Transcriptome analysis using high throughput mRNA sequencing is underway to further characterize the Nkx2.1+ cell population. To test the differentiation potential of the Nkx2.1+ cells *in vitro*, we used co-culture with mouse cortical astrocytes and obtained an enriched population of interneurons in which 75% of the

MAP2+ cells are also GABA+ after 8 weeks. Preliminary studies examining the fate of human ESC-derived Nkx2.1+ progenitors transplanted into the mouse hippocampus demonstrate the expression of neuronal markers 3 weeks post-transplant.

Disclosures: **N.C. Anderson:** None. **C.Y. Chen:** None. **D.F. Moakley:** None. **K. Henderson:** None. **A. Plocik:** None. **B. Graveley:** None. **L. Grabel:** None. **J. Naegele:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: NIH 5T32EY007143

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NIH 5P30EY001765

Research to Prevent Blindness

BrightFocus Foundation

Guerrieri Family Foundation

Mr. and Mrs. Robert and Clarice Smith

Title: Differentiation of human stem cells to retinal ganglion-like cells using a crispr/cas9 engineered reporter line

Authors: *V. M. SLUCH[1], V. RANGANATHAN[2], C. BERLINICKE[2], R. MARTIN[3], H.-Q. MAO[3], D. ZACK[2]
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Abstract: Purpose: A number of diseases lead to retinal ganglion cell (RGC) death and vision loss, the most common of which is glaucoma. Once the optic nerve is damaged, few avenues of intervention exist since the mammalian optic nerve does not regenerate. One ambitious goal is to use cell replacement therapy to restore vision. Human pluripotent stem cell (hPSC)

differentiation could, in theory, provide the necessary RGCs for this strategy. In addition, human stem cell-derived RGCs could make possible an approach for medically relevant drug screening that would have advantages over the current use of rodent RGCs. In addition, such cells could provide insights into human RGC development, gene regulation, and neuronal biology. Here, we describe a protocol for differentiation of hPSCs to RGC-like cells that express a variety of RGC-enriched markers, exhibit spontaneous transient calcium activity typical of neurons, grow projections of over several millimeters *in vitro*, and can be cultured on nanofibers to direct neurite outgrowth. Methods: H7 human embryonic stem cells were genetically engineered using the CRISPR/Cas9 nuclease system to contain a membrane Cherry fluorescent reporter downstream of BRN3B, an RGC-enriched transcription factor. This augmented cell line was differentiated in N2B27 neuronal medium in the presence of 2% Matrigel. Following differentiation, we were able to use fluorescent-activated cell sorting (FACS) to isolate the BRN3B+ RGC-like cells. We analyzed the cells using qPCR, immunostaining, and calcium imaging. Results: By day 30 of differentiation, BRN3B reporter expression was evident, the cells displayed long neurites, and the fluorescent cells could be purified using FACS. The cultures were enriched for BRN3B, BRN3A, SNCG, NEFH, NRN1, and RBPMS expression by qPCR. Additionally, we were able to detect low levels of melanopsin expression. Calcium imaging showed spontaneous calcium activity, typical of neuronal electrical activity. Immunostaining confirmed the presence of cells double positive for MAP2 and BRN3A, neuronal and RGC-enriched markers, respectively. When cultured long term, these cells sprouted neurites of several millimeters in length, and their growth could be directionally guided by aligned nanofibers. Conclusions: We were able to effectively differentiate hPSCs to RGC-like cells. Using CRISPR technology, we generated a stem cell line containing a human RGC reporter, which enables FACS-sorting to obtain isolated RGCs. This study provides a robust method for generating hPSC-derived human RGCs, which can be used for drug screening, developmental and biological studies, as well as cell replacement experiments.

Disclosures: **V.M. Sluch:** None. **V. Ranganathan:** None. **C. Berlinicke:** None. **D. Zack:** None. **H. Mao:** None. **R. Martin:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Program#/Poster#: 397.24/B27

Topic: A.04. Stem Cells

Support: NSF CBET 0939511

Title: A modeling approach to investigate the emergent behavior of motor neurons and glia from 3D stem cell aggregates

Authors: *R. L. SWETENBURG, III[1], D. E. WHITE[2], M. L. KEMP[2], T. C. MCDEVITT[2], S. L. STICE[1]
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Abstract: Progenitor and tissue-specific stem cell domains emerge in development, persist into adulthood and can potentially be harnessed for therapeutic purposes. In the primitive spinal cord, a Sonic Hedgehog (Shh) gradient originating from the notochord and floor plate create distinct progenitor domains in the ventral neural tube. *In vivo*, the motor neuron progenitor (pMN) domain gives rise to motor neurons (MN) early and oligodendrocytes late, both of which are implicated in human health and disease including spinal cord injury and muscular dystrophy. Necessary, but poorly understood, for the preservation of progenitors and this glial switch is Notch signaling, a form of juxtacrine signaling often controlling asymetric division and daughter cell fate in stem and progenitor populations. Pluripotent stem cells (PSC) have the unique ability to form most cells of the body. 3D PSC aggregates, or embryoid bodies (EB), are extremely useful tools for deriving downstream cells types from PSCs. The inherent emergent behavior exhibited by EBs results in heterogeneous, organotypic cell clusters which can be controlled and manipulated with the use of small molecules and growth factors. However, their size, inconsistency of differentiation and heterogeneity make them difficult to interrogate. Using the HBG3 PSC line, which includes a green fluorescent protein driven by a MN specific promoter, we derived MNs and oligodendrocytes from EBs over a 10 day culture period by patterning with retinoic acid and a Shh agonist, purmorphamine. DAPT, a Notch inhibitor, was used to mimic development. To better grasp the spatiotemporal emergence of these cell types, we employed a non-reductionist, evidence-based, computational modeling approach by visualizing pMN progenitors (Olig2+), MNs (GFP+) and oligodendrocytes (Olig2+/Nkx2.2+) with immunocytochemistry. Intact EBs were imaged in suspension using high-resolution, confocal microscopy. A number of metrics were derived from the images for further investigation. Notch inhibition increased the number of MNs, dependent on temporal application of DAPT. DAPT treatment yielded less progenitors based on the inhibitor. The average number of MN clusters was increased in the absence of Notch signaling. Progenitor clustering followed an inverse trend to MN clustering. This data implies that MNs are being produced at the expense of progenitors in the absence of Notch signaling. Further, a principal component analysis show distinct trajectories of the treatments which, along with a computational model, will allow us to make more inferences into how these cells behave in an *in vitro* model and possibly translate to the clinic.

Disclosures: R.L. Swetenburg: None. **D.E. White:** None. **M.L. Kemp:** None. **T.C. McDevitt:** None. **S.L. Stice:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Topic: A.04. Stem Cells

Support: EPA-G2012-STAR-F1

NIH-1R43ES023530-01

Title: Metabolomics and neurite outgrowth as data rich developmental neurotoxicity assays in a pluripotent stem cell derived human neural model

Authors: *A. MAJUMDER[1], S. WALLACE[1], X. WU[2], M. AMOSU[2,3], K. LU[3], M. A. SMITH[2,3], S. L. STICE[1]
[1]Aruna Biomed. Inc., Athens, GA; [2]Regenerative Biosci. Ctr., Univ. of Georga, Athens, GA; [3]Envrn. Hlth. Sci., Univ. of Georgia, Athens, GA

Abstract: A vast majority of chemical entities, ranging from pesticides to compounds of therapeutic interest, remain untested for potential toxic outcomes on human neural development. Current methods for evaluating developmental neurotoxicity (DNT) rely heavily on animal based testing and non-uniform cell lines, are often prohibitively expensive, and provide suboptimal predictive value. To fill this critical gap we evaluated a robust, species representative and physiologically relevant cellular system, combined with high content imaging (HCI) and metabolomics, to address human DNT. Scalable and uniform populations of undifferentiated neural progenitor cells (hNP1TM), differentiated neurons (hN2TM) and astrocytes (hAstroProTM), derived from human embryonic stem cells (hESC) were used to represent a neurodevelopmental continuum and evaluated for toxin induced changes in neurite outgrowth, metabolomic signatures and viability. To address developmental stage specific neurotoxicity, Bis-1, a known neurotoxin, was applied to cultures either during or post neuronal differentiation, as well as in neuron-astrocyte co-cultures to represent neural tissue. Differentiation and neurite outgrowth were measured using HCI. For all of the above, Bis-1 induced reduction in neurite outgrowth were observed at concentrations that did not lower neuronal viability. However, Bis-1 treatment during differentiation selectively reduced the Sox1+ neural progenitor fraction, without affecting more mature HuC/D+ neuronal fraction, suggesting stage specific differences in susceptibilities. Additionally, significant differences in Bis-1 induced toxicity between neuron only and neuron-astrocyte co-cultures were observed, reinforcing the need for better tissue representation in DNT studies. For metabolomics assays cells were treated pre- and post

differentiation, and both media and cells were analyzed by GC-MS. PLS-DA scores plots for Bis-1 show a dose response, and Bis-1 and other toxicants demonstrate class changes and show that changes in metabolomic profiles were induced at doses of test compounds significantly lower than those affecting viability. Thus, like neurite outgrowth, metabolomic profiling provides a more sensitive measure of DNT than a traditional viability assay. Further, it is a data-rich approach for toxic outcomes even when no prior knowledge of compound class or mechanism of action is available. The two approaches provide a powerful advancement in neurotoxicity assessment.

Disclosures: **A. Majumder:** A. Employment/Salary (full or part-time):; ArunA Biomedical Inc.. **X. Wu:** None. **M. Amosu:** None. **M.A. Smith:** None. **K. Lu:** None. **S. Wallace:** A. Employment/Salary (full or part-time):; Aruna Biomedical Inc. **S.L. Stice:** A. Employment/Salary (full or part-time):; Aruna Biomedical Inc..

Poster

397. Neural Differentiation of Pluripotent Stem Cells

Location: Halls A-C

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Topic: A.04. Stem Cells

Support: Smoking Research Foundation Grant

Scientific Research Program from the Japan Society for the Promotion of Sciences 23059321

Title: Involvement of cannabinoid receptors or muscarinic acetylcholine receptors on differentiation into neural progenitor cells in mouse induced pluripotent stem cells

Authors: ***T. ISHIZUKA**, A. OZAWA, M. ARATA, Y. WATANABE
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Abstract: Previous studies reported that anandamide, an endogeneous cannabinoid (CB), promotes differentiation of neural progenitor cells (NPCs). On the other hand, stimulation with muscarinic acetylcholine receptor (mAch receptor) agonists increased differentiation of immature neurons in adult mice. Mouse induced pluripotent stem (iPS) cells display properties of self-renewal and differentiation into various cells including NPCs. The present study examined whether stimulation with either CB receptors or mAch receptors affects differentiation of mouse

iPS cells into NPCs. Mouse iPS cell differentiation was initiated by embryoid body (EB) formation. All-trans retinoic acid (ATRA; 1 microM), HU210 (a CB1 receptor agonist), HU308 (a CB2 receptor agonist), or carbachol (a mAch receptor agonist) was added to the EB cultures for 4 days, and then EBs were transferred to fibronectin-coated dishes and then cultured for 7 days. The differentiation potential from mouse iPS cells into NPCs was evaluated by Nestin expression using immunofluorescence staining and western blot analysis. Treatment with either HU210 (3 nM) or HU308 (3 nM) inhibited ATRA-induced Nestin expression. Pretreatment with rimonabant (0.1 microM; a CB1 receptor antagonist) blocked the inhibitory effect of HU210. Pretreatment with SR144528 (0.1 microM; a CB2 receptor antagonist) also blocked the inhibitory effect of HU308. On the other hand, the treatment with carbachol (10 microM) inhibited ATRA-induced Nestin expression. In addition, the pretreatment with atropine (10 microM) significantly blocked the inhibitory effect of carbachol. Thus, the stimulation with either CB receptors or mAch receptors may inhibit ATRA-induced differentiation of mouse iPS cells into NPCs.

Disclosures: **T. Ishizuka:** None. **A. Ozawa:** None. **M. Arata:** None. **Y. Watanabe:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: A.04. Stem Cells

Support: NIH intramural fund

Title: Differential expression of human endogenous retrovirus-k env in pluripotent stem cells and neural cells

Authors: ***T. WANG**, M. MEDYNETS, E. CHOI, W. LI, A. NATH
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Abstract: Background: Human endogenous retrovirus (Herv) gene sequences occupy almost 7% of the human genome and have been termed “junk DNA”. Amongst them, Herv-K is believed to be the most recently incorporated virus into the human genome. Although incidental Herv-K gene expression has been reported in a variety of pathological conditions, it’s potential expression and role in physiological conditions has not been investigated. Objective: To study the expression and function of Herv-K components in pluripotent stem cells and during the

process of neuronal differentiation. Methods: We used Sendai virus factors containing Yamanaka transcriptional factors to generate induced pluripotent stem cells (iPSC) from human fibroblast cells and CD34 hemopeotic stem cells. The iPSC were further differentiated to neural stem cells and neural cells using induction media. Herv-K expression was detected by RT-PCR, immunostaining and Western-blot assay. The function of Herv-K envelope (Env) was studied by blocking its expression using siRNAs and specific antibodies and then followed by colony formation and morphological studies. The possible interacting ligands of Herv-K Env were studied using co-immunoprecipitation and Western-blot assay. Results: We found that Herv-K components, especially cell membrane Env expression, were increased after iPSC generation compared to the source cells such as fibroblasts and CD34 cells. The expression of Env decreased dramatically after iPSC were differentiated to neural stem cells and neurons. The increased expression of Herv-K was also confirmed in embryonic stem cell line W9. Interestingly, treatment with antibodies against Env resulted in fewer stem cell colonies. When Env expression was blocked by siRNA, there were significant morphological changes in iPSC and ES. Further, Env was co-immuoprecipitated with CD98HC, a protein that plays an important role in cell adhesion and stem cell maintenance. Western-blot assays showed that blocking Env using siRNA resulted in decrease in CD98HC expression. Conclusion: These results indicate that Herv-K Env is expressed in pluripotent stem cells and decreases during neural cell differentiation. It may play an important physiological role in maintaining the pluripotent stem cells by interacting and regulating other factors expressed on stem cells such as CD98HC.

Disclosures: **T. Wang:** None. **M. Medynets:** None. **E. Choi:** None. **W. Li:** None. **A. Nath:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Program#/Poster#: 397.28/B31

Topic: A.04. Stem Cells

Support: DIM Cerveau et Penseé (Region Ile de France)

ENP

MSIF

NMSS

ELA

Title: Neural precursors derived from mouse ips cells extensively remyelinate the demyelinated central nervous system

Authors: S. MOZAFARI[1], C. LATERZA[2], A. MARTEYN[1], C. DEBOUX[1], C. BACHELIN[1], G. MARTINO[2], *A. S. BARON VAN EVERCOOREN[1]
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Abstract: Experimental studies have shown that loss of myelin, results in axonal loss and disability. Furthermore, remyelination can restore lost electrical conductance in demyelinated axons. Finding of an expandable, autologous and reliable source of myelin-forming cells to enhance remyelination is a priority in treating the degenerative component of demyelinating diseases like Multiple Sclerosis (MS). Induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs) have been developed recently from reprogrammed somatic cells. However, the remyelination potential and safety of these cells remain to be well-addressed. The main goal of this study is to fully characterize mouse iPS-NPCs *in vitro* and *in vivo* after transplantation. We first used mouse embryonic neural progenitor cells (mENPCs) as control and characterized iPS-NPCs derived from mouse fibroblasts for their expression of major markers of immature or mature neural cells. Moreover, to investigate the behavior of cells in demyelination context, we induced a focal area of demyelination using Lysolecithin in the spinal cord of adult *ShivererRag* mouse and transplanted the cells in the lesion site 48 hours after demyelination. Animals were sacrificed 1, 2, 6 and 10 weeks post graft to assess survival, migration and differentiation potential of the grafted cells. Our results show that miPS-NPCs similarly to mE-NPCs expressed the immature markers of naturally committed neural progenitor cells *in vitro* at the level of both protein and mRNA transcripts. Moreover, our data from transplantation studies in demyelination context revealed that the grafted iPS-NPCs similar to embryonic cells survived, integrated, timely migrated, and extensively differentiated from immature to mature cells. Grafted cells remyelinated the host axons as confirmed by immunostaining and electron microscopy. Furthermore, no tumor was observed following engraftment. There is a competitive differentiation into bona fide mature myelinating oligodendrocytes. No obvious difference was observed between mE-NPCs vs miPS-NPCs.Our work should help increasing the knowledge about the biology of mouse and in future studies, human iPS-NPCs to establish whether these cells are a useful tool for testing personalized therapies or in regenerative biomedicine in demyelinating diseases like MS.

Disclosures: S. Mozafari: None. **C. Laterza:** None. **A. Marteyn:** None. **C. Deboux:** None. **C. Bachelin:** None. **G. Martino:** None. **A.S. Baron Van Evercooren:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Program#/Poster#: 397.29/B32

Topic: A.04. Stem Cells

Support: Fondation Jérôme LeJeune

Title: Synaptic function of chromosome 21-encoded microRNAs

Authors: *H. MCGOWAN[1], N. MLYNARYK[3], C.-W. LU[2], Z. PANG[2]
[1]Child Hlth. Inst. of New Jersey, Piscataway, NJ; [2]Child Hlth. Inst. of New Jersey, New Brunswick, NJ; [3]Rutgers Univ., New Brunswick, NJ

Abstract: The formation and maintenance of appropriate synaptic connections is a highly regulated process, with misregulation resulting in disordered cognition. Given the emerging information about non-coding RNAs, synaptic functions are likely to be regulated at least in part by these molecules, including microRNAs (miRNAs). miRNAs have been implicated in synaptogenesis, and thus, over- or under-expression of miRNAs in the brain could conceivably contribute to synaptic dysfunction. Human chromosome 21 (HSA21) codes for 5 known miRNAs, and Trisomy 21 (TS21) is the most common genetic form of intellectual disability. Thus, TS21 provides a unique model to study the effect of miRNA overexpression on the functionality of synapses. The objective of this project is to elucidate if and how the overexpression of HSA21 miRNAs leads to dysfunction in synaptic transmission. Furthermore, our preliminary evidence that suggests these miRNAs may affect synaptic integrity via a methyl CpG binding protein 2 (MeCP2)-dependent pathway. Utilizing the induced pluripotent stem (iPS) cell and induced neuronal (iN) cell technologies, we will study the synaptic implications of this miRNA-MeCP2 pathway in human neurons. We have analyzed the 3' UTR of MeCP2 and found putative targeting sites for 4 out of the 5 HSA21 miRNAs, and we have confirmed by dual luciferase assay that they do indeed target MeCP2. In addition, our preliminary findings by qPCR and immunohistochemistry (IHC) suggest that these miRNAs are overexpressed and that MeCP2 is decreased in T21 patient-derived iNs versus control. After further verification of the negative correlation between HSA21 miRNA and MeCP2 expression levels, we will morphologically and functionally characterize the TS21 synapse to support our preliminary electrophysiology data, which suggests impaired synaptic signaling. We will then establish a cause-effect relationship between HSA21 miRNA overexpression and synaptic defects using Tough Decoys to antagonize the miRNAs and "rescue" expression level of MeCP2 and the synaptic function of TS21-iN cells. Furthermore, in order to isolate the synaptic effects of this

miRNA-MeCP2 pathway alone, we will repeat these analyses by overexpressing HSA21 miRNAs in control iNs. Our approach will not only broaden our knowledge of the biological functions of miRNAs, it will also provide insight into mechanistic and molecular bases for the treatment of TS21.

Disclosures: H. McGowan: None. **N. Mlynaryk:** None. **C. Lu:** None. **Z. Pang:** None.

Poster

397. Neural Differentiation of Pluripotent Stem Cells

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Program#/Poster#: 397.30/B33

Topic: A.04. Stem Cells

Support: Lieber Inst. for Brain Development Funding

Title: Transcriptional signatures of lineage bias and human genomic identity in the pluripotent state

Authors: *C. COLANTUONI[1], A. JAISHANKAR[1], G. STEIN-O'BRIEN[1], E. FERTIG[2], J. SHIN[1], S.-K. KIM[1], S. SEO[1], Y. WANG[1], D. HOEPPNER[1], J. CHENOWETH[1], R. MCKAY[1]
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Abstract: In order to reach the goal of personalized medicine, the control and observation of cell types derived from pluripotent human stem cells must achieve a resolution that consistently distinguishes the cellular behavior of individual genomes. Here we show that pluripotent cell lines derived from multiple human genomes have distinct transcriptional dynamics as they self-renew and initiate differentiation. RNA-seq data was obtained from 6 cell lines in conditions that support self-renewal or differentiation to mesendodermal and neuroectodermal fates. Microarray expression data from these same 6 lines and an additional 15 lines was also obtained. Conventional principle component analysis (PCA) of the patterns of transcription within the pluripotent state reveals bias in individual lines that predicts their differentiation efficiency to mes-endoderm or neur-ectoderm. With a novel data analysis approach that incorporates a non-negative matrix factorization algorithm (CoGAPS) we identified individual genome-specific signatures present in pluripotency and in differentiation. These analytic technologies discover stable genome-specific transcriptional dynamics with functional consequences in early human

neural development. This work opens the door to interrogate the cellular mechanisms, genetics, and pharmacology of human brain development from a powerful novel perspective.

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Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.01/B34

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

Support: The Alfred P. Sloan Foundation

The Ray Thomas Edwards Foundation

Princeton Department of Molecular Biology / Princeton Neuroscience Institute

National Institutes of Health (Ruth L. Kirschstein National Research Service Award)

Title: Classical MHCI immune proteins promote synapse elimination at the developing neuromuscular junction

Authors: M. M. TETRUASHVILY[1], M. A. MCDONALD[3], *L. M. BOULANGER[2]
[1]Mol. Biol., [2]Mol. Biol. and Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]U.C. San Diego, San Diego, CA

Abstract: The mammalian neuromuscular junction (NMJ) undergoes prominent developmental synapse elimination, during which supernumerary motor neuron (MN) axons are retracted to produce mature patterns of connectivity. Despite the critical role of synapse elimination in the maturation of motor function, the molecular mediators of this process remain elusive. Specific immune proteins, members of the class I major histocompatibility complex (MHCI), regulate synapse elimination in the developing mammalian visual system, and are highly expressed in adult MNs. We found that MHCI is expressed during synapse elimination at the developing NMJ, and that synapse elimination is impaired in mice genetically lacking cell-surface expression of most MHCI proteins (β2m-/-TAP-/- mice). In these broadly MHCI-deficient mice, significantly more motor end plates remain multiply-innervated relative to WT at postnatal day

15 (P15), when synapse elimination is normally complete. Multiply-innervated NMJs are still apparent in MHCI-deficient adults (P29-60) and aged animals (P365), suggesting that loss of MHCI persistently disrupts synapse elimination at the NMJ. One key unanswered question is which of the dozens of classical and/or nonclassical MHCIs are involved in synapse elimination at the NMJ. We are addressing this question in three ways: (1) by profiling the expression of specific MHCI genes at the NMJ before, during, and after synapse elimination, (2) by determining the extent of synapse elimination in mice lacking the classical MHCI genes, H2-Kb and H2-Db, and (3) by monitoring synapse elimination in mice that overexpress H2-Db specifically in neurons. Since MHCI is upregulated at the NMJ during aging-related denervation, understanding how MHCI contributes to synapse elimination at the developing NMJ could ultimately identify mechanisms of pathological synapse loss during neuromuscular aging.

Disclosures: M.M. Tetruashvily: None. **L.M. Boulanger:** None. **M.A. McDonald:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: CNPQ

FAPERJ

CAPES

PROPPi/UFF

Title: Low omega-3/DHA containing diets delays axonal elimination and the differentiation of cholinergic markers in the rodent visual system

Authors: *C. A. SERFATY[1], P. C. DE VELASCO[2], P. C. SANDRE[3], P. A. RUNG[3], R. M. DOS SANTOS[4], P. C. C. LOPES[3], A. C. F. MELIBEU[3], A. S. FRANCO[2], R. C. A. GUEDES[5], B. A. DA COSTA[5]
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Abstract: Visual subcortical connections develop terminal field specificities through the selective elimination of misplaced axons and the maintenance of correct axonal branches, processes that underlie the use-dependent fine-tuning of topographical maps. In the present work, we studied the impact of a dietary restriction of omega-3 fatty acids on the development of eye specific segregation and topographical specificity of retinofugal pathways. We also studied the impact of low omega-3 diets on the differentiation of retinal layers and cholinergic markers in the retina. Female Lister Hooded rats and their litters were fed with either control (soy oil) or restricted (coconut oil) omega-3 diets. At various postnatal ages, rat pups received eye injections of neuronal tracers to visualize retinal axons at their brain targets. Lipid analysis indicated that the experimental diet led to a selective reduction in DHA content in the visual system. Omega-3 restriction induced an increase in the density of retinal axons in the superficial layers of the SC. This effect was observed throughout the stratum griseum superficiale (SGS), including the ventral and intermediate SGS layers at PND13, PND28 and PND42. The same pattern of expanded terminal fields was observed in the retinogeniculate pathways. The supplementation with fish oil (DHA) for two weeks was able to reverse the abnormal expansion of the retinocollicular projection. We also studied possible mechanisms involved in the loss of synaptic stabilization. Restricted omega-3 groups showed, in the visual layers of the SC decreased levels of GAP-43 phosphorylated form (pGAP-43) consistent with a reduction in synaptic stabilization. Omega-3 restriction increased the content of AMPAR subunits (GluR1 and GluR2) and decreased NMDAR (NR1, NR2A and NR2B) subunits. Omega-3 restricted groups showed a decrease in RXRα content and CREB phosphorylation (pCREB) in the visual layers of the superior colliculus. Retinal differentiation was also affected by omega-3/DHA deprivation. We describe a reduction in rhodopsin immunolabeling and also a reduced labeling of cholinergic markers such as VAchT in the inner nuclear layer and β2-nicotinic receptors in the ganglion cell layer and inner nuclear layer. The data indicate, therefore, that the chronic dietary restriction of omega-3 fatty acids delays axonal elimination, interfering with the maintenance of synaptic connections in the visual system. The data also indicate that the development of the retina is also delayed, specially the development of retinal cholinergic transmission.

Disclosures: C.A. Serfaty: None. **P.C. de Velasco:** None. **P.C. Sandre:** None. **P.A. Rung:** None. **R.M. dos Santos:** None. **P.C.C. Lopes:** None. **A.C.F. Melibeu:** None. **R.C.A. Guedes:** None. **B.A. da Costa:** None. **A.S. Franco:** None.

Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.03/B36

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

Support: NIH Grant R01MH085666

Title: Accelerated gsk-3β activity in prefrontal cortex enhances ltd in a neurodevelopmental schizophrenia model

Authors: *B. XING, W.-J. GAO
Neurobio. & Anat., Drexel Univ. Col. of Med., PHILADELPHIA, PA

Abstract: Schizophrenia is recognized as a neurodevelopmental disorder that results in the emergence of cognitive symptoms during early adolescence. Accumulating evidence suggests that glycogen synthase kinase-3β (GSK3β) play an important role in brain development and synaptic plasticity, and is implicated in schizophrenia. However, it remains unclear how GSK3β changes during critical developmental periods and whether the change involves synaptic plasticity in prefrontal cortex (PFC). Here we show that GSK3β and its serine-9 phosphorylation levels undergo large fluctuations in rat PFC during development and the change of GSK3β activity during this period contributes to the long-term depression (LTD) induction. GSK3β and its inhibitory serine-phosphorylation (Ser 9) in the PFC peaked around the first postnatal week and declined rapidly from the second to sixth weeks of age, when the adult levels were reached. In prenatal methylazoxymethanol acetate (MAM, E17) exposed rats, a neurodevelopmental disruption model of schizophrenia, phosphorylated GSK3β (Ser 9) significantly decreased to adult levels around postnatal day 21 (P21), indicating an accelerated GSK3β activity in the PFC during development. In the saline control animals, LTD could only be induced by low-frequency stimulation with paired pulses in adolescent (~P45) rat medial PFC slices but not in juveniles (P21). Interestingly, the decrease of serine-phosphorylation in the juvenile (P21) MAM-exposed rats appeared to enhance the induction of LTD. In contrast to the saline controls, LTD could be induced in both juveniles and adolescents due to their comparable phosphorylated GSK3β levels. GSK3β inhibitor SB216763 blocks the induction of the age-dependent LTD. These data demonstrate the importance of GSK3β for synaptic plasticity in the PFC, suggesting that abnormal activation of GSK3β signaling during the critical period may contribute to cognitive deficits related to schizophrenia. Supported by NIH R01MH085666 to W.J. Gao.

Disclosures: B. Xing: None. **W. Gao:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: JST PRESTO

KAKENHI

Title: Functional synapse elimination plays a role in large-scale somatotopic refinement in the sensory thalamus of developing mice

Authors: *Y. TAKEUCHI[1], Y. KATAYAMA[1], M. MIYATA[1,2]
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Abstract: Functional synapse elimination and strengthening are crucial developmental processes in the formation of precise neuronal circuits in the somatosensory system, but the underlying alterations in topographical organization are not yet fully understood. To address this issue, we here generated transgenic mice in which afferent fibers originating from the whisker-related brain region, called the maxillary principal trigeminal nucleus (PrV2), were selectively visualized with genetically expressed fluorescent protein. We found that functional synapse elimination drove and established large-scale somatotopic refinement, even after the thalamic barreloid architecture is formed. Before functional synapse elimination, the whisker sensory thalamus was innervated by afferent fibers not only from the PrV2, but also from the brain stem nuclei representing other body parts. Most notably, only afferent fibers from PrV2 onto a whisker sensory thalamic neuron selectively survived and were strengthened, whereas other afferent fibers were preferentially eliminated via their functional synapse elimination. This large-scale somatotopic refinement was, at least partially, dependent on somatosensory experience. These novel results have uncovered a previously unrecognized role of developmental synapse elimination in the large-scale, instead of the fine-scale, somatotopic refinement even after the initial segregation of barreloid map.

Disclosures: Y. Takeuchi: None. **Y. Katayama:** None. **M. Miyata:** None.

Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.05/B38

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

Title: Systematic analysis of the wiring process indicates a vital role for homeostatic control of dendritic excitability

Authors: *P. A. RHODES
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Abstract: The activation of an object-specific pattern in IT 120 ms following retinal activation elegantly implies that wiring embeds cortical function. However, despite decades of experimental discovery detailing individual mechanisms at play in the wiring process, including axonal search and competition, dendritic spikes and other local events that contribute to synaptic disintegration and stabilization, these mechanisms have not yet been synthesized to build predictive understanding of the self-organization of neural circuits. To begin a systematic program addressing this goal simulations were developed incorporating the mechanisms enumerated above, and the wiring process quantified during the presentation of parameterized artificial sensory input. An array of branched nonlinearly activated neurons was innervated by a population of axons representing the output of a simulated visual or olfactory sensor array. Upon formation each synapse was associated with an initial stability value, with a set of eight independent wiring parameters defining the increment or decrement of stability that occurred upon the joint conjunction or disjunction of axonal, branch and cell firing. When this stability value declined below a floor the connection was eliminated and the newly vacant dendritic location innervated by a randomly redrawn axonal partner. In this system the unsupervised self-organization of wiring unfolded in time. In some parameter regimes the initially random wiring first gave way to a period where each branch was dominated by axons associated with a distinct feature (i.e. a set of correlated inputs), so that cell firing initially signified the joint presence of at least a threshold number of co-active features. If but only if cell firing per se furnished additional stability reward, then competition and cooperation between the various branches gradually resulted in a common feature innervating all of them, with neuronal output coming to signify the presence of just one feature (i.e. a simple cell). The results also robustly indicated that wiring self-organization required an intermediate level of branch firing: not only did too little activity fail to elicit the rewards and penalties that prompted selective synaptic disintegration, but too much activity also prevented self-organization from occurring. The cell and branch firing rates within which selective wiring developed was observed to be between 1% and 10%. These results support the conjecture that the information-rich self-organization of neural wiring likely requires the homeostatic regulation of branch excitability that is found ubiquitously in neural systems.

Disclosures: **P.A. Rhodes:** A. Employment/Salary (full or part-time):; Evolved Machines.

Poster

398. Activity-Dependent Changes in Connectivity

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Program#/Poster#: 398.06/B39

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

Support: MRC DOCTORAL TRAINING GRANT STUDENTSHIP

Title: Formation of rich clubs in neuronal functional networks *in vitro*

Authors: *M. S. SCHROETER[1], P. CHARLESWORTH[2], M. KITZBICHLER[1], O. PAULSEN[2], E. BULLMORE[1]
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Abstract: Recent studies demonstrated that the structural backbone of the human brain follows a "rich-club" organization. This complex topological feature implies that highly connected regions, hubs of the large-scale brain network, are more densely inter-connected with each other than with nodes of lower degree. Demonstrating high centrality, rich-club nodes were also traversed by a majority of short-paths between regions, underlining their potential importance for efficient global exchange of information between functionally specialized areas of the brain. Though hub neurons have already been described at the micro-scale of brain connectivity, their role in shaping functional synchronous activity and forming microcircuit wiring during development is not yet fully understood. The present study aimed at investigating the role of hubs during network development *in vitro*, using multi-electrode local field potential coupling during spontaneous neuronal activity of dissociated primary hippocampal neurons. Emergence of functional connectivity is demonstrated by a significant increase in average connectivity strength and network size over the first 4 weeks *in vitro*. Furthermore, we found a pronounced rich-club coefficient in more mature networks. Rich-club connectivity is central to the functional network: Results show that rich-club nodes connect early in development, demonstrating structural similarities to network models grown with a preferential-attachment, "rich-get-richer", rule. Moreover, rich-club nodes of mature networks play an important role as leader or broker for initiating and shaping spontaneous activity in the network. Cascades of spontaneous activity preferentially started from, or traversed via, rich-club nodes, but not from nodes of the periphery. These results potentially indicate that the rich club topology of hub nodes may play an important role in coordinating functionally specific dynamics at the local microcircuit level.

Disclosures: **M.S. Schroeter:** None. **M. Kitzbichler:** None. **P. Charlesworth:** None. **O. Paulsen:** None. **E. Bullmore:** Other; Brain Mapping Unit, Department of Psychiatry, University of Cambridge, Cambridge, UK, Clinical Unit, GlaxoSmith Kline, Addenbrooke's Centre for Clinical Investigations, Cambridge, UK.

Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.07/B40

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

Support: Wellcome Trust

EPSRC

Title: Detecting pairwise correlations in spike trains: an objective comparison of methods and application to study of retinal waves

Authors: ***C. CUTTS**, S. J. EGLEN
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Abstract: We present a computational study objectively comparing measures for quantifying pair-wise correlations in neuronal spike times. We use a detailed case study of the developing retinotopic map to demonstrate the benefits of our results. In this case study, we make use of existing published data. Quantification of the degree of correlations between recorded neuronal spikes is a key part of analysis of experimental data in a wide range of systems. Correlations in neuronal spike times are thought to play a key role in information processing since the output of an individual neuron is small and unreliable therefore several neurons may be required for reliable processing. One example is the developing retinotopic map, where correlations in spontaneous retinal activity have been implicated in its formation. In this field, recordings are made on multi-electrode arrays and the correlations calculated pair-wise across the array. Correlations are then compared against the separation of the electrodes where the neurons were recorded and this is then compared, along with the associated retinotopic map, across different genotypes. From this, inferences are made about the role of correlated activity in map formation. Many measures have been proposed to summarise these correlations and there is a standard measure used to quantify correlations in spontaneous retinal activity - the Correlation Index (Wong et al Neuron, 1993). We show that the Correlation Index is unbounded above and

confounded by firing rate. We specify properties which a measure should have in order to fairly measure correlation. We then propose a novel measure, the Tiling measure. This measure is then tested, together with 34 previously published measures, blindly and extensively for the required properties. On the basis of this testing and analytical analysis, we propose a measure to replace the Correlation Index. We re-analyse data from key studies which investigated the role of spontaneous retinal activity in map formation and show that re-analysis of the data using a measure of correlation which is independent of firing rate can significantly change the conclusions.

Disclosures: C. Cutts: None. **S.J. Eglen:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: UCSB Academic Senate grants

Title: Prenatal serotonin levels can affect the development of the prefrontal control of the dorsal raphe nucleus

Authors: S. E. GROSS, A. CHEN, V. F. LU, M. B. GREENBERG, *S. JANUSONIS
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Abstract: The dorsal raphe nucleus (DRN) is a major source of serotonin (5-HT) in the brain and it is controlled by several forebrain regions. The DRN receives a major projection from the medial prefrontal cortex (mPFC). This projection has been implicated in several mental disorders associated with serotonergic dysfunction (autism spectrum disorders, major depressive disorder, schizophrenia, and others). The activity of DRN-projecting neurons in the mPFC is regulated by a number of neurotransmitter receptors, among which three serotonin receptors (5-HT1A, 5-HT2A, 5-HT4) directly affect their firing rates. We have shown that prenatal perturbations of 5-HT levels result in altered 5-HT4 receptor expression in the embryonic telencephalon, and that some of these effects are mRNA splice variant-specific (Chen et al., 2012). Recently, we have found that acute prenatal exposure to fluoxetine (a selective serotonin reuptake inhibitor) decreases the mRNA levels of the 5-HT1A, 5-HT2A, and 5-HT4 receptors in the embryonic telencephalon (Chen and Janusonis, submitted). Taken together, these findings suggest that

abnormal 5-HT levels in prenatal development can affect the activity of mPFC neurons that send their axons to the DRN. This, in turn, may disrupt the activity-dependent formation of mPFC-DRN synapses and lead to a dysfunctional prefrontal control of behavior later in life. In this study, we investigated (i) whether prenatally-induced changes in the expression of serotonin receptors persist in the adult mPFC and (ii) whether a decrease in the expression of one serotonin receptor (5-HT4) during development can affect the structure of mPFC-DRN synapses. In the first part of the study, timed-pregnant C57BL/6 mice were exposed to fluoxetine, and the mRNA levels of the 5-HT1A, 5-HT2A, and 5-HT4 receptors were analyzed in the mPFC of adult offspring with quantitative reverse-transcription PCR (qRT-PCR). In the second part, the density of mPFC-DRN synapses was investigated in mice with a null-mutation in the 5-HT4R gene (B6.129P2-Htr4(tm1Dgen)/J). The mPFC terminals in the DRN were labeled by using the vesicular glutamate transporter 1 (vGluT1) as the marker, and synapses were quantified with confocal microscopy and an algorithm for automated colocalization detection (Costes et al., 2004). Adult 5-HT4+/- mice (but not 5-HT4-/- mice) had a significantly higher density of synaptic contacts between mPFC terminals and 5-HT-positive neurons in the ventromedial DRN. It suggests that a prenatally induced decrease in the expression of some serotonin receptors may result in permanent changes in the prefrontal control of brain serotonin signaling.

Disclosures: **S.E. Gross:** None. **A. Chen:** None. **V.F. Lu:** None. **S. Janusonis:** None. **M.B. Greenberg:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: NIH 5R01NS031651

Title: Role of cyclic AMP signaling in synaptic refinement at the *Drosophila* NMJ

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

Abstract: Neural activity plays a key role in synaptic plasticity and refinement. In the vertebrate visual system, low frequency calcium and cyclic nucleotide oscillations refine early topographic maps. At the *Drosophila* neuromuscular junction (NMJ) refinement also occurs in an activity-

dependent manner. Oscillatory neural activity and presynaptic calcium signaling modulate the motoneuron's response to the retrograde chemorepellent Sema-2a for the removal of off-target synapses in a CaMKII-dependent fashion. In addition, mutations of the calcium-dependent adenylyl cyclase rutabaga (*rut*), and the cAMP phosphodiesterase dunce (*dnc*) increase the frequency of miswired NMJs. We find that in *rut* mutants 60% of the ectopic synapses are from an octopaminergic type II motoneuron, while 40% arise from type I motoneurons. By contrast, in *dnc* mutants more than 80% are from the octopaminergic neuron. RNAi knockdown of *rut* in neurons phenocopies the frequency and the distribution of the ectopic contacts in *rut* mutants, indicating a presynaptic role of *rut* in synaptic refinement. However, the miswiring phenotype observed in *rut* mutants can be partially rescued by expressing rut in either neurons or muscles, indicating that *rut* signaling is also involved in a retrograde signal from the muscle. Overexpression of rut and dnc in either neurons or muscles also significantly increases the frequency of ectopic synapses, suggesting that cAMP levels are tightly regulated (both high and low) on both synaptic sides. We have also observed increased frequency of ectopic synapses after neuronal RNAi knockdown of the regulatory subunit R1 of PKA, a target of cAMP. As rut is dependent on Ca activity, we are testing whether cAMP levels must also oscillate for normal refinement. For live imaging experiments we use embryos mutant for the *mhc1* gene to suppress movement, monitoring Ca activity with GCaMP5. Low frequency (0.01Hz) Ca oscillations are evident in both native and ectopic contacts. We are also testing whether cAMP must oscillate by using the photoactivated adenylyl cyclase bPAC to rescue *rut*. At moderate expression levels we can suppress ectopic contacts in *rut* mutants following a protocol that mimics the native Ca oscillation (15 sec on:150 sec off); no rescue is observed without bPAC activation. We are also using the FRET based cAMP sensor epac1-camps as a tool for *in vivo* imaging of endogenous cAMP levels at motoneuron growth cones. These results show that as in vertebrates, synaptic refinement in *Drosophila* is dependent on dynamic signal transduction mechanisms that engage both Ca and cyclic nucleotide oscillations.

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

Poster

398. Activity-Dependent Changes in Connectivity

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

Title: A role of calcium influx through GluN2B receptors in the critical period plasticity for corticospinal synapse elimination

Authors: *T. OHNO[1], N. ISOO[1], M. ISOWAKI[1], S. FUKUDA[1], M. MISHINA[2], M. SAKURAI[1]
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Abstract: Neuronal plasticity is generally active in young age and some of them decline or disappear after a short time window so called "critical period". Once the period is closed, loss of plasticity limits modifiability of function in adulthood and it will never be re-opened throughout life. Molecular mechanisms for such temporal restriction on neuronal plasticity, however, still remains poorly understood. It is well known in thalamic and cortical synapses that shift of NMDA receptor subunit composition from GluN2B (2B) to GluN2A (2A) occurred during early postnatal development, raising possibility that this shift is involved in the closure of critical period. However, the involvement of NMDA subunit shift in ending critical period is questioned in barrel and visual cortex. In this study, utilizing *in vitro* slice co-culture model of corticospinal projection system that shows 2B-dependent synapse elimination possessing critical period, we tried to identify specific biological mechanism that closes the critical period. In order to selectively activate the corticospinal axons, we infected the cortical slices with AAV-CaMKII-hChR2 (H134R)-EYFP and optogenetically stimulated with LED light (465 nm). This method also allows live imaging of EYFP labeled- corticospinal axons. We also employed calcium imaging to evaluate the amount of calcium influx through 2B channels. Slices were stained with calcium-sensitive dye, fluo-4 AM and optical measurements of fluorescence changes of the dye were performed using high speed camera system. We first confirmed the presence of 2B-2A-shift in cultured spinal cord, that is, synaptic 2B declined markedly toward the end of the critical period. In 2A knockout mice that express 2B at high level throughout life showed elongation of critical period. Up-regulation of synaptic 2B in wild type mice by proBDNF or spermine application re-opened the once closed critical period. As opposed to synaptic 2B, glycine receptors in the spinal cord gradually increase during postnatal development accoding to *in vivo* study. Partial reduction of inhibitory input by strychnine, which enhanced the amount of Ca influx through 2B channels without up-regulating synaptic 2B, also extended the critical period suggesting that the development of inhibitory inputs also play an important role in determining the critical period. Our findings, however, indicate that decline of 2B plays a pivotal role in closing the plastic time window and the development of inhibitory input might also be an important player in regulation of critical period length through modulation of 2B activity.

Disclosures: T. Ohno: None. **N. Isoo:** None. **M. Isowaki:** None. **S. Fukuda:** None. **M. Mishina:** None. **M. Sakurai:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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Program#/Poster#: 398.11/B44

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

Title: Defining the role of neurotrophins in the neonatal hippocampus

Authors: A. JALLOH[1], *B. J. CATLOW[1], D. A. PAREDES[1], L. GRAND[1], J. CHUNG[2], T. Y. N. NG[2], R. D. MCKAY[1]
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Abstract: During the period of programmed neuronal death in the neonatal hippocampus (HPC), neurotrophins (NT) trigger neuronal activity and this activity promotes neuronal survival through Akt signaling. Later in development, Akt is no longer rate-limiting but a MAPK/STAT3 pathway is required for circuit formation. In this study, levels of the NT brain derived neurotrophin factor (BDNF) and neurotrophin-3 (NT3) were measured in dissected HPC throughout post-natal (P) development using ELISA. In addition, antibodies against phospho epitopes of Akt, MAPK, and STAT3 were used to map the ontogeny of expression in the developing rat HPC by immunofluorescence and western blot analysis. Clear increases in BDNF and NT3 occur both in early P development and later after the first P week. Akt, MAPK, and STAT3 show distinct patterns of developmental expression in the HPC, which corroborate the role of early Akt activity and a later requirement for STAT3. To analyze the electrophysiological consequences of BDNF exposure, *in vitro* slice recordings were performed at P4 and P6 on the HPC of rat pups. 64 channel Multielectrode Arrays recorded single neuronal extracellular spiking activity from the HPC. This approach showed that BDNF immediately increased the frequency of action potential firing of HPC neurons on P4. Application of the same BDNF dose 2 minutes after the first administration changed the firing mode of neurons from single units to bursting mode, suggesting intrinsic change of the neurons. To define the effects of BDNF *in vivo*, multichannel extracellular recordings from HPC areas were obtained with a 4-shank multichannel silicon probe implanted into the P2 and P7 HPC of wild type and BDNF+/- knock-out rats. Significantly increased HPC oscillatory activities were found in all frequency ranges of P7 compared to P2 neonates. Beta and low-frequency gamma oscillation power showed the most significant increase, most prominently in CA3. Increased single unit firing activity of CA1 and CA3 neurons characterized gamma bursts and were related to micro muscle twitches during active sleep. These data show neuronal activity elicits two distinct phases of second messenger responses in the post-

natal hippocampus. The mechanism of genetic risk associated with psychiatric disorders has been modeled by systems that model early events in dissociated neuronal cell culture, in the system we report, neuronal activity can be controlled by delivering exogenous compounds (including NT) or by physiological inputs to the post-natal hippocampus *in vivo*. This approach opens new ways to link disruption in the early steps of neuronal functional maturation to later global dysfunction of brain circuits.

Disclosures: A. Jalloh: None. **B.J. Catlow:** None. **D.A. Paredes:** None. **L. Grand:** None. **J. Chung:** None. **T.Y.N. Ng:** None. **R.D. McKay:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: HHMI

Nancy Lurie Marks Family Foundation

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Title: Neuromodulatory state-dependent plasticity in striatal development

Authors: *Y. KOZOROVITSKIY, R. PEIXOTO, B. SABATINI
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Abstract: Dopamine signaling is poised to influence the development of striatal excitatory drive, because dopaminergic fibers are present in the striatum long before most excitatory synapses are formed. Among its many actions, dopamine impinges upon intracellular cascades implicated in cellular and circuit development, such as the activity of Protein Kinase A (PKA). We found that *in vivo* activation of D1 type dopamine receptors (D1Rs) rapidly increased the number of dendritic spines and synapses on direct pathway spiny projection neurons (dSPN), and this effect required PKA activity. To distinguish cell-autonomous and circuit-level requirements for synaptogenesis, we induced and monitored the growth of new dendritic spines using 2-photon glutamate uncaging, while imaging SPN dendrites in acute brain slices. We found that activation of Gs-coupled receptors was sufficient to enhance synaptogenesis. Finally, we utilized an

optogenetic strategy to demonstrate the relevance of this PKA-dependent, fast plasticity form in the developing corticostriatal circuits *in vivo*.

Disclosures: Y. Kozorovitskiy: None. **R. Peixoto:** None. **B. Sabatini:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

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FIRST program

MEXT KAKENHI (22115009)

Title: Cell-autonomous function of connexin 36 in dendritic refinement of barrel cells during postnatal development

Authors: *W. LUO[1,2], H. MIZUNO[1,2], S. ITOHARA[3], T. IWASATO[1,2]
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Abstract: In immature neocortex, the expression of connexin 36 (Cx36), a neuron-specific gap junction protein, and electrical coupling between cortical neurons increase during first two weeks of postnatal development, and then decline during the following two weeks, when the number of chemical synapses increase most rapidly. Besides these characterized developmental profiles, whether and how gap junction participates in neural circuit formation remains unclear. To address this issue, we utilized whisker-barrel circuit as a model system. In layer 4 (L4) of mouse somatosensory cortex, spiny stellate neurons (barrel cells) extend their dendrites toward barrel center to make synapses with thalamocortical axon terminals. This dendritic asymmetry, which is established during early postnatal stage in an activity-dependent manner, enables each barrel cell to process information from one whisker. Here, we characterized the role of gap junction in whisker-barrel circuit formation, especially the role of Cx36 in dendritic refinement of barrel

cells. Firstly, we blocked endogenous Cx36 function by transfecting dominant negative form of Cx36 (mutant Cx36) into most or sparse L4 neurons. Morphological analysis under both conditions showed that, in barrel cells over-expressed with mutant Cx36, the dendritic length inside the barrel was smaller than that in control barrel cells, while the dendritic length outside the barrel was rarely different. These results indicate that Cx36 functions cell-autonomously to regulate dendritic refinement of barrel cells by enhancing their dendritic orientation through extending their inside dendrites. We also over-expressed mutant Cx36 into most L4 neurons from 2nd postnatal week, after barrel cell dendrites acquired orientation bias. Histological analysis revealed that the inside dendritic length in barrel cells which loose functional Cx36 from 2nd postnatal week was smaller than that in control barrel cells, indicating that Cx36 may function from 2nd postnatal week to regulate dendritic refinement of barrel cells. Further analysis will be performed to characterize the molecular and cellular mechanisms of Cx36-dependent dendritic refinement of barrel cells.

Disclosures: W. Luo: None. **H. Mizuno:** None. **S. Itohara:** None. **T. Iwasato:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: EY11261

Title: Topographic map plasticity by a Spatial-Temporal-Spatial transformation of sensory input

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Abstract: Topographic maps are spatial representations of the sensory environment that are located throughout the CNS and are thought to allow efficient computation within brain circuits. Current models do not provide an adequate explanation for the role of activity in map formation. Using the visual system of Xenopus, we show that the spatial distribution of retinal ganglion cells axons in the CNS is specified by the temporal order of retinal ganglion cell activity in a colinear manner. Spatial information in the visual scene is transformed into the temporal sequence of activation of retinal ganglion cells. Subsequently the temporal sequence of activity in retinal cells is transformed into the spatial arrangement of afferents in the target. The Spatial-

Temporal-Spatial (STS) transformation ensures that topographic projections are scaled across available target area.

Disclosures: M. Hiramoto: None. **H. Cline:** A. Employment/Salary (full or part-time):; The Scripps Research Institute.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: NIMH MH095229

Beatrice and Samuel A. Seaver Foundation

Simons Foundation

Title: Role of Sema7A in maturation of sensory cortical circuits

Authors: *N. KEZUNOVIC, I. CARCEA, T. AHMED, R. MESIAS, P. BURMAN, H. MORISHITA, J. D. BUXBAUM, G. W. HUNTLEY
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Abstract: Early sensory experience exerts profound control over cortical circuit maturation during an early 'critical period' (CP) of heightened cortical plasticity. Disturbances in CP circuit maturation are thought to contribute to cognitive and psychiatric disorders. The molecules that promote circuit maturation during CPs are poorly understood. Sema7A is an atypical member of the Semaphorin family that is GPI-linked, is expressed principally postnatally, and is enriched in sensory cortical areas during the CP. Accordingly, we investigated the role of Sema7A in circuit maturation in mouse somatosensory (barrel) and visual cortex. We found that Sema7A is highly expressed by L4 spiny stellate cells and GABA neurons. In barrel cortex, genetic ablation of Sema7A disrupts barrel cytoarchitecture and impairs the generation of appropriately oriented L4 spiny cell dendrites. Thalamocortical axon ingrowth to L4 is unaffected. Additionally, Sema7A ablation leads to imbalances in the ratio of excitation-to-inhibition evoked by thalamocortical input to L4, which is likely due to delayed maturation of GABAergic inhibition. Levels of GAD65/67 are lower and labeling of GABA-circuit markers is sparser in both S1 and V1 of Sema7A knockout mice compared with wildtype (WT) mice. In V1, it is known that the onset of

GABA inhibition is important for initiating the CP for ocular dominance (OD) plasticity. Thus, we predicted that delayed GABAergic inhibition in V1 of Sema7A KO mice would affect OD plasticity. As expected, brief (4d) monocular deprivation (MD) during the CP failed to alter OD in the absence of Sema7A, indicating that mutant V1 circuits are insensitive to experience-dependent maturation. In humans, microdeletions in 15q24, which include SEMA7A, lead to autism and sensory perceptual deficits. Together, these data suggest that Sema7A is a critical molecular mediator of experience-driven local circuit maturation during the CP, deficits in which may lead to autism and related cognitive and perceptual abnormalities.

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Poster

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

Support: Wellcome Trust Career Development Fellowship

Royal Society Research Grant

Title: An optogenetic screen for temporal spike patterns reveals an instructive role for neuronal activity in plasticity at the axon initial segment

Authors: M. D. EVANS, A. S. LOWE, *M. S. GRUBB
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Abstract: Neuronal activity is vital in many aspects of brain development. In most cases it is known to be ‘permissive’, where a certain level of spiking is required for a particular maturational or plastic event to occur. In far fewer cases is it known that neuronal activity is ‘instructive’, with particular patterns of spiking sculpting particular developmental outcomes. The gold standard for this permissive versus instructive distinction is to compare the effects of activity patterns that differ in their spatial or temporal structure while maintaining equal overall firing rates. However, it has proven difficult to control ongoing neuronal activity with sufficient scope or precision to fully explore the dependence of any particular phenotype on specific patterns of spiking. Here we took advantage of optogenetic technology to undertake an

exhaustive screen of temporal patterns in spike firing, mapping the activity pattern space associated with plasticity at the site of action potential initiation, the axon initial segment (AIS). We sparsely transfected dissociated hippocampal neurons with a channelrhodopsin-2-YFP construct that rendered them light sensitive. At 10 days *in vitro* we pharmacologically blocked circuit-driven spontaneous activity, then employed 3h chronic photostimulation using custom-built LED arrays. Individual flash stimuli were of 5ms duration at an intensity known to produce reliable spiking in our transfected neurons. Inter-stimulus intervals (ISIs) were drawn randomly from a negative binomial distribution, accounting for all second-order structure in a stimulus train by describing temporal patterns in terms of two independent parameters: mean ISI, or 'rate', and the squared coefficient of variation of ISI, or 'burstiness'. Cultures were subsequently fixed, stained for cell-type markers and the AIS scaffolding protein ankyrin-G, imaged, and quantitatively analysed for AIS length in dentate granule cells (DGCs). We found clear evidence that the frequency of stimulation alone cannot account for activity-dependent changes in DGC AIS length. A 2-dimensional distribution of AIS length according to pattern rate and burstiness displayed a clear 'hotspot' where particular combinations of input frequency and temporal structure were capable of reducing the length of the AIS over a 3h time period. Our optogenetic activity screen therefore demonstrates the instructive nature of neuronal activity in inducing length changes at the AIS, and goes further by precisely demarcating the input pattern parameters that produce this form of activity-dependent plasticity.

Disclosures: **M.D. Evans:** None. **M.S. Grubb:** None. **A.S. Lowe:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: CSIR GRANT 37(1420)/10-EMR-II

Title: Early chronic loud noise exposure alters the metabolic profile and expression of synaptic proteins in the developing chick auditory cortex analogue

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Abstract: Development of the functional (tonotopic) organization and signal processing capabilities in the auditory cortex (ACx) critically depend on the early sensory experiences. With inevitable increase in the exposure to chronic high intensity sound, it has become a major public health concern, especially for fetuses and neonates. Here we report the effects of chronic loud noise exposure (110 dB) during the embryonic development of chick (Gallus gallus domesticus) on the metabolic profile and synaptic proteins of the auditory cortex analogue (AuL). Fertilised eggs were exposed to loud noise from embryonic day 10 until hatching. Expression pattern of the synaptic proteins were studied by immunohistochemistry, western blotting and qPCR and metabolic profiling by 700 MHz 1H NMR spectroscopy of post hatch day-1 auditory cortices. Protein expression analysis showed a significantly reduced expression of synaptophysin, PSD 95, gephyrin and GABA-A γ-2 subunit but an enhanced expression of AMPA GluA-2 subunit in the noise exposed group. Multivariate regression analysis (Principal Component Analysis and Partial Least Square-Discriminant Analysis) of the concentration data of 18 metabolites quantified through NMR spectroscopy showed a separate clustering of the control and noise exposed samples. Statistical analysis (R2=0.95, Q2=0.88) showed a difference in metabolic profiles between the two groups suggestive of a metabolic perturbation following chronic loud noise exposure. Noise exposure significantly reduced the levels of GABA, energy metabolites-glucose, β-hydroxybutyrate, NAD and ATP and neuromodulators aspartate and taurine. The levels of glutamate and glutamine were significantly higher in noise exposed group as compared to control. Overall the prenatal chronic loud noise exposure increased the excitatory to inhibitory (E/I) neurotransmission related component ratio and reduced the expression of synaptic stability and plasticity maker in the AuL. Increased excitation and poor inhibition not only alter the spectrotemporal and spatial encoding of different sound frequencies but also lead to excitotoxicity, as indicated by the reduced levels of N-acetyl aspartate, thus explaining the decreased AuL neuronal number reported in our earlier stereological study (Sanyal, et al., 2013. Int. J. Dev. Neurosci.). Depleted levels of major energy metabolites indicated an enhanced neuronal activity due to chronic exposure to high intensity sound. Thus early chronic loud noise significantly alters the E/I ratio and metabolic profile which affects the functional development of the auditory cortex and associated behavior (Sanyal et al., 2013. PloS One).

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Poster

398. Activity-Dependent Changes in Connectivity

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

Support: CIHR

Savoy Foundation

CHU Sainte-Justine

Title: Early-life febrile seizures induce a precocious expression of KCC2 and impairment of excitatory synapse formation

Authors: *P. N. AWAD[1,2], B. CHATTOPADHYAYA[1], N. SANON[1], J. SZCZURKOWSKA[3], E. BAHO[1,2], J. NUNES CARRIÇO[1], S. DESGENT[1], L. CANCEDDA[3], L. CARMANT[1,2], G. DI CRISTO[1,2]
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Abstract: Febrile seizures affect about 5% of children during the first year of life. Atypical febrile seizures, particularly febrile status epilepticus, correlate with a higher risk of developing cognitive deficits and temporal lobe epilepsy as adults, suggesting that they may permanently change the developmental trajectory of neuronal circuits. In fact, the presence of a cerebral malformation predisposes to the development of atypical febrile seizures and temporal lobe epilepsy. The mechanisms underlying these effects are not clear. Cation-chloride cotransporter KCC2 decreases intracellular Cl^- levels and renders GABA responses hyperpolarizing. Recent data suggest that KCC2 also modulates excitatory synapse development. Here, we demonstrated that KCC2 expression is altered by early-life febrile status epilepticus and investigated the functional impact of this alteration on subsequent synapse formation. We analyzed KCC2 expression and spine density in the hippocampus of a well-established rodent model of atypical febrile seizures, combining a cortical freeze lesion at post-natal day 1 (P1) and hyperthermia-induced seizure at P10 (LHS rats). 86% of these LHS males develop epilepsy and learning and memory deficits in adulthood. At P20, we found a precocious increase in KCC2 protein levels, accompanied by a negative shift of EGABA following high-frequency stimulation. In parallel, we observed a striking reduction in dendritic spine density and of mEPSC amplitude and frequency in CA1 pyramidal neurons. To investigate whether KCC2 precocious overexpression plays a role in spine alterations, we mimicked it in hippocampal organotypic cultures by biolistic transfection and *in-vivo* by *in-utero* electroporation. We found that both manipulations decreased spine density. Finally, to causally link KCC2 increased expression to spine loss in the LHS model, we blocked KCC2 *in vivo* by *in utero* electroporation of shRNA, and induced the dual pathologies as explained above. We are currently investigating whether reducing KCC2 expression levels in LHS rats rescues spine density loss. Our results show so far that an increase

of KCC2 levels induced by early-life seizure seem to affect spine formation and may be a contributing factor to the occurrence of hippocampal atrophy and associated cognitive deficits in LHS rats. Funded by CIHR, Savoy foundation and CHU Sainte-Justine Foundation.

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Poster

398. Activity-Dependent Changes in Connectivity

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Program#/Poster#: 398.19/B52

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

Support: Savoy Foundation

Canada Research Chair

Title: The role of Tsc1 in the development of cortical GABAergic connectivity

Authors: ***M. CHOUDHURY**[1,2], J. N. CARRIÇO[1], M. BERRYER[1,2], G. DI CRISTO[1,2]
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Abstract: The mTOR pathway has been implicated in controlling several aspects of neurodevelopment by regulating the rate of protein-synthesis. Mutations in the regulatory components Tsc1 and Tsc2 of mTOR-Complex1 (mTORC1) cause Tuberous Sclerosis (TSC) in humans. The majority of TSC patients develop neurological problems including seizures, mental retardation and autism. Recent studies investigated the role of mTOR pathway dys-regulation in excitatory cortical cells, however its role in the development of cortical GABAergic interneurons and the specific contribution of altered GABAergic cells in disease manifestation remain largely unknown. Here, we investigated whether and how Tsc1 knockout perturbs GABAergic circuit development, both *in vitro* and *in vivo*. We found that pS6 immunolabeling, a marker of mTORC1 activation, increased specifically in cortical Parvalbumin-positive, basket GABAergic cells (BCs) during the peak of their synaptic maturation phase, between the 2nd and the 4th postnatal week postnatal *in vivo*. To investigate the role of mTORC1 activation in BC development, we knocked down Tsc1 expression, by transfecting CRE-GFP driven by a

promoter specific for BCs in cortical organotypic cultures prepared from Tsc1flox mice. Tsc1 knockdown *in vitro* caused a precocious increase in bouton density and terminal branching formed by mutant BCs, which was reversed by Rapamycin treatment. These data suggest that mTOR pathway hyperactivation affects the timing of BC synapse maturation. To investigate the role of mTORC1 in GABAergic cells *in vivo*, we bred Tsc1lox mice with Nkx2.1-CRE mice. Nkx2.1 is expressed by GABAergic neurons derived from the medial ganglionic eminence, which include BCs. At P18, Tsc1fl/fl::Nkx2.1Cre mice showed both mTORC1 hyperactivation in BCs and increased expression of VGAT, a presynaptic GABAergic marker. These data suggest that BCs may form boutons prematurely in these transgenic mice. Behavioral studies are currently underway to investigate possible deficits in working memory and social behavior.

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Poster

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

Support: Canada Research Chair Program

CFI

Title: Role of SYNGAP1 in GABAergic synapse development

Authors: *M. H. BERRYER[1], B. CHATTOPADHYAYA[2], J. ANTOINE-BERTRAND[3], N. LAMARCHE-VANE[3], F. HAMDAN[4], G. DI CRISTO[2], J. MICHAUD[2]
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Abstract: SYNGAP1, which codes for a Ras GTPase-activating protein (GAP), is a component of the NMDA receptor complex. We have previously shown that heterozygous loss-of-function mutations in SYNGAP1 cause nonsyndromic intellectual disability (NSID) with or without epilepsy and autism. Constitutive knockdown of Syngap1 in mice results in a hyperactivation of Ras, increase of ERK phosphorylation, excess number of GluR1 at the surface of post-synaptic

membrane, altered dendritic spine development and impaired long term potentiation associated with cognitive and behavioral deficits. Several recent studies have shown that impairment of glutamatergic synapse development contribute to the cognitive phenotype in Syngap1+/- mice. Whether Syngap1 affects GABAergic cell synapse development, thus contributing to excitation/inhibition imbalance and cognitive deficits, is unknown. Here, we show that Syngap1 is expressed by GABAergic neurons. We further show that single-cell knockout of Syngap1 in basket cells, a prominent subtype of GABAergic neurons, in cortical organotypic cultures affects GABAergic axon branching and perisomatic synapse formation. To dissect the role of Syngap1 in GABAergic cell development *in vivo*, we generated conditional knockout mice by breeding Syngap1flox/flox with Tg(Nkx2.1-Cre) mice, which express CRE exclusively in GABAergic neurons derived from the medial ganglionic eminence. We are currently characterizing the cognitive and social behavior of Tg(Nkx2.1-Cre);Syngap1flox/+ mice versus Syngap1+/- mice. All together, our data suggest that Syngap1 modulates GABAergic synapse development. The behavioral phenotype associated with Syngap1 haploinsufficiency may thus be caused at least in part by a decrease of its expression in inhibitory neurons.

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Poster

398. Activity-Dependent Changes in Connectivity

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

Support: NIH RO1 NS081297-01

Title: Dynamic changes in the connectivity of MGE-derived cortical interneurons during postnatal development

Authors: ***S. N. TUNCDEMIR**[1], F. J. STAM[2], F. OSAKADA[2], M. GOULDING[2], E. CALLAWAY[2], G. FISHELL[1]
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Abstract: The complex functions of the cerebral cortex rely on highly connected networks of excitatory neurons, integrated together by a diverse population of GABAergic cortical interneurons (cIN). Assembly of this network involves a series of events that are co-regulated by

genetic programs and neuronal activity. Yet, an understanding of how diverse cIN populations incorporate into cortical circuitry has lagged behind. Low threshold-spiking SST and fast-spiking PV cINs are the two most abundant cINs subtypes in the cortex derived from the medial ganglionic eminence (MGE), and largely populate the (early born) deep cortical laminae. Although previous studies have ascribed a latent maturation of FS-PV cINs; it is increasingly clear that during early postnatal development, SST cINs contribute to the emergence and synchronization of network activity. Using monosynaptic rabies virus tracing and optogenetics, we found significantly denser thalamic inputs (TC) to the L5/6 SST cINs during the first postnatal week. In contrast, deep layer SST cINs in juvenile mice receive only weak TC inputs; yet receive dense intracortical input from layer 5/6 and layer 2/3 pyramidal neurons (PyrN). Interestingly, we found that immature SST-cINs receive strong monosynaptic TC inputs compared to adjacently positioned FS-PV cINs and PyrNs in L5/6, which are rapidly destabilized at the end of the first postnatal week, resulting in a shift in the TC feed-forward inhibitory drive from SST cINs to PV-cINs. We suggest that maturation of TC feed-forward inhibition is associated with exuberant transient functional connectivity onto SST cINs that is later pruned to achieve predominantly FS-PV mediated feed-forward inhibition in the adult. As such, we hypothesize that dynamic patterns of TC input onto two distinct cIN types directly regulate their incorporation into the cortical network.

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Poster

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

Support: MSCRF

Title: Interplay between GABAergic local circuitry and DISC1 in regulating synaptic integration of newborn neuron in adult hippocampus

Authors: E. KANG[1], J. SONG[2], Y. GU[3], S. GE[3], K. M. CHRISTIAN[4], B. BERNINGER[5], H. SONG[1], *G.-L. MING[1]
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Sch. of Med., Baltimore, MD; [5]Univ. Med. Ctr. of the Johannes Gutenberg Univ., Mainz, Germany

Abstract: Schizophrenia etiology is thought to involve the interaction between genetic and environment factors during brain development. Despite significant progress in understanding both genetic susceptibility and neuronal circuit dysfunction in schizophrenia, fundamental gaps exist in our knowledge about how circuitry mechanisms may interact with genetic susceptibility to affect neuronal development. Disrupted-in-schizophrenia 1 (DISC1), a risk gene for major mental disorders, regulates various processes of neuronal development, including adult hippocampal neurogenesis, deficiency of which alone is sufficient to cause cognitive and affective behavior deficits. We found that DISC1 knockdown-induced glutamatergic synapse formation defects of newborn neurons in the adult hippocampus required GABAergic input-induced depolarization. Rabies virus-mediated retrograde tracing and optogenetic analyses further identified aberrant GABAergic synaptic inputs from parvalbumin-expressing (PV+) and somatostatin-expressing (SST+) interneurons. Functionally, optogenetic suppression of SST+ neuron activation rescued dendritic defects of DISC1-deficient newborn neurons, but not glutamatergic synapse formation. In contrast, suppression of PV+ neuron activity partially rescued DISC1 deficiency-induced glutamategric synaptic formation defects, but not aberrant dendritic development. Therefore, distinct neuronal circuit interacts with the genetic susceptibility to manifest different aspects of aberrant neuronal development.

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Poster

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

Support: MH051234

MH084053

Title: Postnatal development of parvalbumin-positive GABA neurons in mouse prefrontal cortex

Authors: *G. GONZALEZ-BURGOS, T. MIYAMAE, T. TIKHONOVA, D. A. LEWIS
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Abstract: Parvalbumin-positive (PV) GABA neurons significantly shape neocortical circuit development and are crucial regulators of neural activity in mature cortical cell networks. Comparing the development of the two main PV neuron subtypes, basket cells (BCs) and chandelier neurons (ChNs), is thus essential to understand the roles of ChNs and BCs in regulating the emergence of cortical network activity. Furthermore, distinctive developmental trajectories of ChN and BC function would suggest that PV neuron subtypes could be differentially affected by environmental factors that increase the risk of mental illnesses such as schizophrenia. Recent studies suggested that relative to BCs, ChNs originate later in development. If so, then developing ChNs may achieve a mature functional state later than BCs, unless ChN function develops at a faster rate. To distinguish between these possibilities, we began studying the postnatal development of PV neuron function in mouse prefrontal cortex (PFC). We performed targeted patch clamp recordings from GFP-labeled neurons in brain slices prepared from the PFC of G42 mice, a mouse strain in which GFP is expressed exclusively in PV neurons. The recorded neurons were filled with biocytin to characterize their morphology post-hoc. As suggested in previous studies, we found that as early as postnatal day 15 (P15) biocytin-filled PV neurons could be classified as ChNs or BCs based on the presence or absence, respectively, of characteristic vertical cartridges of axonal boutons. In current clamp recordings, we examined the intrinsic properties of the recorded neurons measuring multiple parameters, including input resistance (Rin), action potential (AP) threshold, AP duration at half maximal amplitude, after hyperpolarization (AHP) amplitude and a spike frequency adaptation coefficient (SFA). These parameters were measured from PV neurons of P15 to P45 mice, and the trajectory of change was fit with exponential functions. Some parameters did not change with age (AP threshold, AHP), but others exhibited a similar type of change in BCs and ChNs. For instance, in both ChNs and BCs, Rin, AP duration and SFA decreased significantly with age until reaching a plateau. However, preliminary curve fit analysis showed that, consistent with a later developmental origin of ChNs, the plateau state was reached later in ChNs compared with BCs. These preliminary results indicate that in PFC circuits the intrinsic excitability of PV neurons, which is crucial for activity-dependent development of PV neuron signaling, matures following different time courses in BCs and ChNs.

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Poster

398. Activity-Dependent Changes in Connectivity

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3 K99 MH095825-02S1

National Institute of Mental Health

National Institute of Neurological Disorders and Stroke

New York Stem Cell Science State

Simons Foundation

Title: Activity-dependent cortical interneuron circuit formation

Authors: *T. KARAYANNIS, R. PRIYA, S. TUNCDEMIR, G. J. FISHELL, N. V. DE MARCO GARCIA
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Abstract: Neuronal microcircuits within the superficial layers of the mammalian cortex provide the cellular substrate for associative cortical computation. Inhibitory interneurons constitute an essential component of the circuitry and are fundamental for the integration of local and long-range information. Nevertheless, the mechanisms by which these interneurons integrate into the developing circuit are not known. Using a monosynaptic rabies-based approach, optogenetics and electrophysiology, we report that, during early development, superficially positioned Reelin-expressing neurogliaform interneurons in the somatosensory cortex receive innervation from both cortical and thalamic excitatory populations. Interestingly, the inputs from the latter seem to predominantly activate NMDA receptors subtypes that are necessary for driving the proper axo-dendritic morphological development of neurogliaform cells, as well as for setting the balance between their intracortical and thalamic inputs. These results reveal that sensory-driven activity shapes the assembly of specific cortical interneuron circuits.

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Poster

398. Activity-Dependent Changes in Connectivity

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

Support: NIH R01DC009607

Title: Excitatory input to cortical GABAergic interneurons during development

Authors: *R. DENG[1], P. O. KANOLD[2]
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Abstract: GABAergic interneurons are important for shaping sensory computation in mature brain, but are also important in regulating the critical period during brain development. During development, neural activity contributes to the maturation of GABAergic neurons, but the source of this activity on GABAergic neurons is unknown. Excitatory inputs onto GABAergic neurons could contribute to this activity. During the early developmental period, subplate-neurons (SPN) are the first population of neurons exhibiting mature electrical properties and participate in transient circuits relaying neural activity from thalamus to excitatory cortical neurons. Since SPN ablation prevents the maturation of GABAergic function we hypothesize that SPN may provide excitatory input to GABAergic neurons during brain development and thereby aid in the maturation of GABAergic neurons. We use a mouse expressing gad2cre driven red fluorescence protein to identify cortical GABAergic interneurons in the middle cortical layers of mouse auditory cortex. We then investigate the presynaptic sources of glutamatergic input to these GABAergic interneurons by laser-scanning photo stimulation (LSPS) during postnatal development (P5 to P13). We find that a fraction of gad2cre labeled GABAergic interneurons receive excitatory input from SPN and the fraction of GABAergic interneurons receiving excitatory input from SPN was larger in older mice (P9 to P13) during the period of GABAergic maturation and during the critical period than in neonatal mice (P5 to P7). Thus, excitatory inputs from SPN might regulate neuronal activity dependent development of GABAergic interneurons by providing excitatory input in early development. Therefore, removal of SPN's input to GABAergic neurons in early development removes this excitation and thereby might prevent GABAergic maturation, leading to cortical hyperexcitability.

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Poster

398. Activity-Dependent Changes in Connectivity

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

Support: NIH Training Grant 5T32GM007377-35

NSF Grant 0845285

Title: Enrichment of PSD-MAGUKs in nascent dendritic spines

Authors: *J. T. LAMBERT, T. C. HILL, K. ZITO
Univ. of California, Davis, Davis, CA

Abstract: Animals have a remarkable capacity to change their behavior to suit a wide range of circumstances. At the cellular level, this behavioral flexibility is made possible because individual neurons modify their connectivity in response to varying patterns of synaptic activity in a process termed experience-dependent plasticity. During experience-dependent plasticity, the selective stabilization of new dendritic spines is linked tightly with functional changes in neural circuits. High-frequency stimulation that induces long-term potentiation (LTP) has been shown to increase the stability of new spines, but the molecular mechanism of this activity-dependent structural stabilization is ill-defined. Here, we begin to characterize the molecular composition of new spines compared to mature spines. We used time-lapse two-photon imaging of CA1 pyramidal neurons expressing DsRed-Express and EGFP-tagged postsynaptic proteins, focusing on the membrane associated guanylate kinases (MAGUKs) PSD-93, PSD-95, SAP-97, and SAP-102. We assessed the relative enrichment of these postsynaptic proteins in new spines compared to spines that were present and stable throughout the imaging session. We found that all four PSD-MAGUKs are less enriched in new spines compared to their persistent neighbors. This difference is a function of spine age, not of spine size, as persistent spines were similarly enriched regardless of size. Intriguingly, PSD-MAGUKs enriched in new spines at different rates. GFP-SAP-97 and GFP-SAP-102 enriched within 3 hours of spine outgrowth ultimately reaching levels similar to neighboring, persistent spines. Notably, GFP-PSD-93 is unenriched in new spines, but enrichment increases gradually, reaching mature levels by 6 hours. GFP-PSD-95 did not enrich in new spines over the entire course of the time-lapse (7.5 hours). Current experiments are focused on characterizing the effects of stabilization-inducing activity on the enrichment of PSD-MAGUKs in new spines.

Disclosures: **J.T. Lambert:** None. **T.C. Hill:** None. **K. Zito:** None.

Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.27/B60

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

Support: NIH Grant DC012095

Title: Experience-dependent axon targeting and guidance molecule expression in mouse olfactory system

Authors: *N. N. GONG[1], H. MATSUNAMI[2]
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Abstract: Olfactory sensory neurons (OSNs) that express the same olfactory receptors (ORs) are scattered in the olfactory epithelium, yet their axons coalesce into just a few bundles of axons, or glomeruli, on each olfactory bulb, a process that is still not fully understood. Previous work indicates that both activity-dependent and –independent mechanisms are involved in OSN axon fasciculation and targeting, but whether olfactory experience plays a role is unclear. This study aimed to determine the effect of early postnatal odor experience on expression of axon guidance molecules and subsequent OSN axon targeting, focusing on two related ORs, M71 and M72, that normally converge into nearby yet separate glomeruli on the olfactory bulb. First, mice were exposed to acetophenone, a cognate ligand for both receptors, for 16-hours per day from the day of birth until the mice were 21 days old. Strikingly, stimulation affected M71- and M72- OSNs so that the axons of these OSNs coalesced onto the same glomerulus. Double-label fluorescent *in situ* hybridization was then used to compare the expression levels of known axon guidance molecules, including Kirrel2, Kirrel3, EphA5, ephrinA5, and BIG-2, in OSNs. The data suggest postnatal stimulation with acetophenone influences expression of certain axon guidance genes involved in OSN targeting: in mice stimulated with acetophenone, M71-OSNs exhibited significant downregulation of Kirrel3 expression, while in M72-OSNs, Kirrel2, ephrinA5, and BIG-2 expression were significantly downregulated. Next, the relationship between OR responsivity of M71, M72 and a series of M71/M72 chimeras *in vitro* and published axon targeting data *in vivo* of OSNs expressing these ORs was examined. A significant correlation was found between OR responsivity *in vitro* and glomerular targeting *in vivo*, further supporting our hypothesis that ligand-dependent OR activation has a critical role in determining axon targeting. Together, our study reveals an experience-dependent component involved in OSN targeting in the olfactory bulb.

Disclosures: **N.N. Gong:** None. **H. Matsunami:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

Support: French Ministry of Research fellowship

ANR Jeunes Chercheurs

Fondation pour la Recherche Medicale

Retina France

Université Pierre et Marie Curie

INSERM

Title: Differential requirement of presynaptic release for eye-specific and topographic retinal maps

Authors: *A. REBSAM[1], A. ASSALI[1], M. BENNIS[2], P. KAESER[3], T. C. SÜDHOF[4], P. GASPAR[1]
[1]INSERM U839, Inst. du Fer à Moulin, Paris, France; [2]Cadi Ayyad Univ., Marrakesh, Morocco; [3]Harvard Med. Sch., Boston, MA; [4]Howard Hughes Med. Institute, Stanford Univ., Stanford, CA

Abstract: Projections of Retinal Ganglion Cells (RGCs) in their main targets, the dorsal Lateral Geniculate Nucleus (dLGN) and the Superior Colliculus (SC), are organized in eye-specific domains and with precise topography. Projections are initially intermingled and are refined into their final territories during the first postnatal weeks. Alteration of spontaneous neural activity in the retina disrupts both eye-specific segregation and retinotopy. However, the cellular mechanisms linking neural activity to map refinement remain poorly understood. Here we examine the role of presynaptic release on the refinement of retinal projections. To distinguish between the synaptic and non-synaptic effects of activity blockade, we perturbed specifically the presynaptic release at retinal terminals by a conditional deletion of Rim 1 and 2 in RGCs. The removal of Rim proteins is known to strongly reduce calcium-dependent neurotransmitter

release, without affecting spontaneous release. Our tracing studies indicated that Rim conditional double knock-out (Rim cDKO) mice have defects in eye-specific segregation in the dLGN but no major topographic defects in the SC. This result suggests that segregation but not gross topography involves calcium-dependent synaptic release. Interestingly, ipsilateral projections in the SC do not form patches and extend more laterally in Rim cDKO compared to control mice, suggesting that ipsilateral projections could be more sensitive to the perturbed presynaptic release. Our results show that retinal synaptic release is important for eye-specific segregation and for the organization of ipsilateral projections.

Disclosures: **A. Rebsam:** None. **A. Assali:** None. **M. Bennis:** None. **P. Kaeser:** None. **T.C. Südhof:** None. **P. Gaspar:** None.

Poster

398. Activity-Dependent Changes in Connectivity

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

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Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

National Key Basic Research Program of China (2013CB530900)

Title: Activity-dependent regulation of liprinα1 during synapse development

Authors: *H. HUANG[1,2,3], K.-O. LAI[1,2,3], A. FU[1,2,3], N. IP*[1,2,3]
[1]Div. of Life Science,HKUST, Hong Kong, China; [2]Mol. Neurosci. Center, HKUST, Hong Kong, China; [3]State Key Lab. of Mol. Neuroscience, HKUST, Hong Kong, China

Abstract: The liprinα family proteins, originally identified as interacting protein of the receptor protein tyrosine phosphatase LAR (LAR-RPTP), are suggested to play an indispensable role in dendrite development and formation of excitatory synapse. However, the regulation and function of liprinα1 in neural development remains unclear. We found that liprinα1 was highly expressed at the post-synaptic fractions during early postnatal stages, when activity-dependent synapse development actively occurs. Furthermore, liprinα1 was identified as an *in vivo* substrate of Cdk5, and this phosphorylation was regulated in cultured neurons by neuronal activity.

Interestingly, phosphorylation of liprinα1 in the mouse visual cortex was also modulated during eye opening, suggesting that liprinα1 phosphorylation might be involved in activity-dependent synapse development *in vivo*. Importantly, knockdown of liprinα1 in cultured hippocampal neurons led to defects in dendritic arborization and reduction in spine density. Together, our results reveal a novel mechanism of activity-dependent synapse development that involves phosphorylation of liprinα1.

Disclosures: H. Huang: None. **K. Lai:** None. **A. Fu:** None. **N. Ip*:** None.

Poster

398. Activity-Dependent Changes in Connectivity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 398.30/C3

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

Title: Expression analysis of genes encoding transcription factors, mbo-1, 2, 3 in oryzias latipes, medaka

Authors: *R. WATANABE[1], S. YOKOI[1], Y. ISOE[1], S. ANZAI[2], M. KINOSHITA[2], A. UGAJIN[3], T. OKUYAMA[1], T. KUBO[1], H. TAKEUCHI[1]
[1]Univ. of Tokyo, Tokyo, Japan; [2]Kyoto Univ., Kyoto, Japan; [3]Tamagawa Acad., Tokyo, Japan

Abstract: In various animal species, some instinct behaviors, such as mating behaviors and social behaviors, dynamically alter in an age-dependent manner. It has been well-known that endocrine systems regulate the behavioral and physiological alteration according to age. However it remains largely unknown how neural reshaping contributes the behavioral alteration. To approach this issue, we have focused on a transcription factor, Mblk-1, which is a possible candidate that is responsible for neural reshaping. mblk-1 was originally identified in honeybee as a gene specifically expressed in the higher-order brain center (mushroom body), which is associated with behavioral alteration according to age-dependent division of labor in the workers (Takeuchi et al., Insect Mol. Biol. 10, 487-494, 2001). Furthermore a nematode homologue of Mblk-1 (mbr-1) is required for the neural reshaping (pruning), which occurs from the larval to adult stages (Kage and Hayashi et al., Curr. Biol. 15, 1554-1559, 2005). Although vertebrate homologues of Mblk-1 are also identified, their roles in the nervous system have not been studied. We began to investigate the vertebrate homologue using medaka fish, because medaka fish exhibit remarkable behavioral alteration according to their age (Imada et al., PLoS ONE, 5, e11248, 2010), and detail neuronal morphology is observable in the medaka brain. Here we

report identification of cDNAs for three medaka homologues of Mblk-1 (Mbo-1, 2, 3) and their expression analysis. First qRT-PCR using various adult tissues showed that the medaka homologues were expressed mainly in the nervous system and/or reproduction organ. *In situ* hybridization showed that mbo-1 was expressed prominently at the habenula and the preoptic area, which are suggested to be involved in emotional and reproductive behaviors. Now we generated mbo mutant strains using CRISPR/Cas system to examine whether these homologues are involved in the neural reshaping and behavioral alteration.

Disclosures: **R. Watanabe:** None. **S. Yokoi:** None. **Y. Isoe:** None. **S. Anzai:** None. **M. Kinoshita:** None. **A. Ugajin:** None. **T. Okuyama:** None. **T. Kubo:** None. **H. Takeuchi:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.01/C4

Topic: A.08. Transplantation and Regeneration

Title: Injury-induced decline of intrinsic regenerative ability revealed by quantitative proteomics

Authors: ***S. BELIN**[1], H. NAWABI[1], C. WANG[1], P. WARREN[2], H. SCHORLE[3], C. UNCU[2], Z. HE[2], J. STEEN[2]
[1]Neurol., [2]Pathology, Boston Childrens Hosp., Boston, MA; [3]Dept. of Developmental Pathology, Univ. of Bonn Med. Sch., Bonn, Germany

Abstract: The inability of the mature central nervous system (CNS) to regenerate suggests a loss of function in the injury response associated with aging. Intense focus has been directed toward understanding the mechanisms responsible for the regenerative failure of adult neurons in the mammalian CNS, and on overcoming such failure. A particular emphasis was placed on the role of the inhibitory environment and the role of development-dependent mechanisms, which. identifying several molecular regulators. However, manipulations of these pathways result in limited axon regeneration *in vivo*, suggesting that other major molecular factors are yet to be discovered. In this study, our hypothesis was that the injury response in the adult CNS inhibits the regenerative capacity of retinal ganglion cells (RGCs). Thus we systematically characterized injury-triggered responses RGCs. We performed a quantitative proteomic analysis of a pure fraction of neurons obtained after FACS sorting YFP positive RGC before and after optic nerve injury. Using bioinformatics analyses, we generate a map of cellular functions and pathways specifically regulated by injury. In addition, we obtained a list of potential upstream regulators

potentially essential for neuron survival and regeneration. While our study reveals a network of injury-response signaling hubs, including known regeneration regulators such as mTOR, calcium, and MAPK, novel molecules were also identified. We focused on one of the new potential regulators, the c-myc transcription factor, which is down regulated by axotomy. c-myc appears to be an important regulator that orchestrates both neuronal survival and axon regeneration in RGCs. Forced expression of c-myc in RGCs, either before or after injury, promotes RGC neuronal survival and axon regeneration. As our protemics study highlighted the simultaneous alteration of several pathways by injury, we decided to overexpress c-myc in a context of PTEN and Socs3 deletion. Surprisingly, modulating this combination of factors dramatically increases axon regeneration in the model of optic nerve injury with a substantial increase in the number of axons entering and crossing the chiasma. Altogether, our results describe the first quantitative proteomics of a pure RGC *in vivo* population, revealing the major effect of injury in CNS neurons. Our study highlights several new potential key players of neuron survival and axon regeneration which might represent novel targets for developing neural repair strategies in the adult CNS.

Disclosures: **S. Belin:** None. **H. Nawabi:** None. **C. Wang:** None. **P. Warren:** None. **C. Uncu:** None. **Z. He:** None. **J. Steen:** None. **H. Schorle:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.02/C5

Topic: A.08. Transplantation and Regeneration

Title: Musashi 1 expression in regenerating axolotl retina

Authors: *E. A. DEBSKI, J. MAYNOR, M. SINDALL
Univ. of Kentucky, Lexington, KY

Abstract: The axolotl salamander is able to repair damage to its retina caused by injury. We have been examining the process by which it does so following optic nerve and ophthalmic artery transection. These manipulations induce a severe degeneration of the retina that reduces this structure to a rudiment within1-2 weeks. Over the course of the next 6-8 weeks, the lost cells are replaced and the neural circuitry is re-built. To help identify stem cell populations involved in this process, we have been examining patterns of Musashi 1 expression during the regeneration process. Musashi 1 is a RNA-binding protein that is expressed in the retina in both

stem/progenitor cells and some differentiated cell types (Kaneko and Chiba (2009) Neurosci Lett. 450:252-257). In central sections of the control retinas of juvenile axolotls, Musashi 1 immunoreactivity is reliably found in a subset of small cells within the retinal pigment epithelium and surrounding cell bodies in the outer nuclear layer. In contrast to reports in other species, the outer nuclear layer staining is not contained within the cell bodies of photoreceptors. Rather, it appears to be continuous with occasionally observed staining in Müller radial glia and thus most likely represents the apical ends of these cells. In lateral retinal sections, glial staining with Musashi 1 can continue into the inner plexiform and retinal ganglion cell layers. Three days after retinal injury, Musashi 1 immunoreactivity extends to a large area of the retinal pigment epithelium. While glial cell immunoreactivity is indistinguishable from that seen in controls, bright irregular shaped spots of immunoreactivity are seen throughout the retinal layers. By one week after injury, Musashi 1 positive cells with the large inclusions characteristic of macrophages are seen along the inner limiting membrane. Macrophage-like Musashi 1 positive cells are abundant by the time that the retina is reduced to a rudiment and Müller glial cell staining is maintained in a manner consistent with their changed morphology at this stage. We are in the process of extending our observations to later time points when cells are being born to rebuild the retina. However, our results to date indicate that Musashi 1 may play an important role in the injury response resulting in retinal degeneration. Furthermore, the maintenance of Musashi 1 expression in Müller radial glial at a time of tremendous cell death suggests that it may help ensure the survival of these cells and/or be needed in the subsequent repair process.

Disclosures: **E.A. Debski:** None. **J. Maynor:** None. **M. Sindall:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.03/C6

Topic: A.08. Transplantation and Regeneration

Title: Rubrofugal projections: Novel mediators of spontaneous recovery after spinal cord injury

Authors: *C. S. SIEGEL, S. M. STRITTMATTER, W. B. CAFFERTY
Neurol., Yale Univ., New Haven, CT

Abstract: Spinal cord injury (SCI) results in irreversible devastating functional impairments. Damaged axons fail to regenerate after SCI because of the inhibitory environment in the mature central nervous system (CNS) and the low intrinsic growth capacity of adult neurons. As an

alternative to regeneration, plasticity of intact circuits represents a potent route to recovery of function after SCI. Previous data from our lab demonstrated that the intact corticospinal tract (CST) could functionally sprout into the denervated side of the spinal cord in the absence of nogo receptor 1 after unilateral pyramidotomy (uPyX). We sought to determine whether sprouting of intact circuits could restore function after complete CST lesion. To test this, adult wild type (n=20) and *ngr1*$^{-/-}$ (n=20) mice received bilateral pyramidotomies (n=10 bPyX, n=10 sham/genotype). Mice were behaviorally assessed via grid walking, fore limb tape removal and footprint analysis pre-surgically and on days 2, 4, 7, 14, 21, 28 and 35-post lesion. Seven days before the end of the experiment, mice received Biotinylated Dextran Amine (BDA) microinjections into their left side Red Nucleus to trace rubrofugal projections, and Alexa-Fluor488-conjugated Dextran microinjections into their right side sensorimotor cortex to trace the corticofugal projections rostral to the lesion. Wild type mice demonstrated persistent deficits throughout the experimental period, while *ngr1*$^{-/-}$ mice demonstrated a trend toward enhanced functional recovery. The density of intact cortico-rubro, cortico-pontine, rubro-pontine, rubro-raphe, and rubro-spinal projections were enhanced in all lesioned animals compared to sham. *ngr1*$^{-/-}$ mice demonstrated significantly more dense projections between these nuclei in comparison to wild type mice. To dissect the impact of these *de novo* circuits on functional recovery after bPyX we used pharmaco-genetics to silence each circuit. Preliminary data shows that AAV-mediated overexpression of the inhibitory DREADD (Designer receptors exclusively activated by designer drugs) hM4Di receptor into the nucleus raphe magnus abrogates functional recovery in *ngr1*$^{-/-}$ mice after bPyX. Therefore, we hypothesize that a novel rubro-raphe circuit is capable of restoring function after complete axotomy of the CST and is thus a new anatomical substrate to target therapies to treat severe SCI.

Disclosures: C.S. Siegel: None. **S.M. Strittmatter:** None. **W.B. Cafferty:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.04/C7

Topic: A.08. Transplantation and Regeneration

Title: Identification of novel modulators of intrinsic CNS axon growth

Authors: *K. FINK, S. STRITTMATTER, W. CAFFERTY
Yale Univ., New Haven, CT

Abstract: Axons do not grow after spinal cord injury (SCI) because the central nervous system (CNS) is an inhibitory growth environment and adult CNS neurons have a diminished intrinsic growth capacity. Numerous studies have reported that relief of environmental inhibition by nullifying the effects of myelin associated inhibitors (NogoA, MAG and OMgp) and chondroitin sulfate proteoglycans (CSPGs), results in enhanced, yet clinically unsatisfactory, axon growth and functional recovery after SCI. Elevating the growth capacity of CNS neurons has been more challenging owing to a paucity of molecular targets. We sought to identify novel intrinsic modulators of axon growth by completing differential transcriptomic analysis between corticospinal tract neurons in an active or quiescent growth state after unilateral pyramidotomy (uPyX). Transgenic *crym-gfp ngr1*$^{+/+}$ (n=6) and *crym-gfp ngr1*$^{-/-}$ (n=6) mice received uPyX followed two weeks later by unilateral intraspinal injection of the retrograde tracer fast blue (FB) into the denervated cervical cord. Four weeks after lesion, mice were prepared for laser capture microdissection and RNA extraction. *Crym-gfp* mice express soluble GFP in CST neurons allowing us to identify this population at high density and fidelity. Neurons that had sprouted arbors into the denervated side of the spinal cord take up FB. Therefore, CST neurons were identified in cortex as sprouting (GFP+FB+) or non-sprouting (GFP+FB-) and isolated using laser capture microdissection. CST neurons were also collected from uninjured perinatal mice (P5) and adult mice (P28) to compare CST sprouting to active and quiescent growth states, respectively. Laser captured cells were prepared for RNA sequencing and sequenced to an average depth of 52 million reads per sample with an average of 66% mapping uniquely to genes. Transcriptional profiles were compared using TopHat and Cufflinks. 1174 genes were significantly differentially expressed between intact sprouting and non-sprouting neurons. Functional relevance of significantly differentially expressed genes was explored using Ingenuity Pathway Analysis. Functional analysis revealed 30 phosphatases that are differentially regulated in sprouting neurons. Of these phosphatases, 10 were knocked down in an *in vitro* cortical neuron scrape assay using shRNA to assess their effects on growth. The assay revealed that knockdown of 5 of the 10 candidate phosphatases significantly impaired growth of cortical neurons *in vitro*. Ongoing studies will reveal if these phosphatases can be targeted *in vivo* to enhance plasticity and regeneration after spinal cord injury.

Disclosures: K. Fink: None. **S. Strittmatter:** None. **W. Cafferty:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: NIH Grant R01NS064288

The Craig H. Neilsen Foundation

Title: Targeting non-muscle myosin II to promote axon regeneration after optic nerve injury

Authors: *X. SAIJILAFU[1], Y. JIAN[2], Y. GUO[2], C. LIU[2], J. JIANG[2], M. ZHANG[2], F. ZHOU[2]
[1]Johns Hopkins Med. Sch., Baltimore, MD; [2]Departments of Orthopaedic Surgery, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Axon regeneration is essential for the restoration of neuronal connectivity and functional recovery after nerve injury. However, axons in the adult mammalian central nervous system (CNS) have very limited growth ability following injury, due to the inhibitory environment and diminishing intrinsic axon growth capacity. Our previous cell culture study has found that inhibition of nonmuscle myosin II (NMII) with either pharmacological inhibitor or siRNAs drastically increases axon growth of multiple neuronal types over either extracted CNS myelin or chondroitin sulfate proteoglycans (CSPGs), two major categories of inhibitory molecules in the CNS. However, it is unknown whether inhibition of nonmuscle myosin II have similar effects on axon regeneration *in vivo*. Herein, we showed that deletion of endogenous NMIIA/B in mouse retinal ganglion neurons significantly enhanced axon regeneration after the optic nerve crush injury without affecting the neuronal survial. Lens injury is well known to promote the axon regeneration of retinal ganglion neurons after optic nerve injury. We also found that lens injury and deletion of endogenous NMIIA/B act synergistically to promote enhanced axon regeneration. Thus, we believe that regulation of the local cytoskeletal machinery in the growth cone would provide an alternative and novel therapeutic treatments for peripheral and central nerve injuries.

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Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: CIHR

The Foundation for Fighting Blindness

Title: Inhibition of BMP and sFRP2 proteins in the adult mouse eye induces proliferation and expands the retinal stem cell population

Authors: *K. N. GRISE, L. BALENCI, D. VAN DER KOOY
Mol. Genet., Univ. of Toronto, Toronto, ON, Canada

Abstract: Adult retinal stem cells (RSCs) are a rare subset of cells that reside in the pigmented ciliary epithelium (CE) of the mammalian eye. Once dissociated from the CE, RSCs readily proliferate to form clonal, free floating spheres after 7 days, with the capacity to self-renew and differentiate into all of the cell types of the neural retina and retinal pigmented epithelium (RPE). Despite having the capacity to proliferate *in vitro*, RSCs do not proliferate or generate new retinal cells in adult mammals *in vivo*. Thus, we aimed to identify factors responsible for maintaining RSC quiescence and determine if those factors can be targeted *in vivo* to disinhibit RSC proliferation and induce retinal neurogenesis. To investigate if discrete tissue types in the eye contribute to the inhibitory RSC niche, we generated tissue-specific conditioned media (CM) from postnatal day 2 and adult mouse eyes and added it during the primary RSC clonal sphere formation. Only CM from the adult lens and cornea reduced the number of spheres formed. We considered two proteins known to be expressed in the adult eye as potentially mediating the lens and cornea CM inhibition: bone morphogenic proteins (BMPs) and secreted frizzled related protein 2 (sFRP2). We found that BMP and sFRP2 proteins could dose-dependently impede sphere formation. When noggin (BMP inhibitor) and α-sFRP2 antibody were added in combination to lens and cornea CM, sphere number returned to control levels. Thus, inhibiting BMP and sFRP2 signaling eliminated lens and cornea CM-induced quiescence of adult RSCs *in vitro*. Next, we investigated whether BMP and sFRP2 inhibition could disinhibit RSC quiescence *in vivo*. We injected noggin or α-sFRP2 intravitreally into the right eye 3 times; once every 24 hours. The left eye was injected with an equivalent volume of PBS as a control. All injections also included 0.5ug/uL of EdU. Seven days after the last injection, EdU-positive cells were detected in both noggin and α-sFRP2 treated eyes at a much higher frequency than control at all doses. Also, the number of EdU-positive cells in both conditions appeared to be dose-dependent. Further, by performing a clonal sphere assay 6 days after injection, we found that α-sFRP2 doubled the number of primary RSC spheres. These results establish that inhibition of BMP and sFRP2 signaling can cause proliferation within the adult mouse eye. Also, inhibiting sFRP2 appears to expand the retinal stem cell pool. Experiments are now underway to better characterize the cell types being induced to proliferate, the combinatorial effects of noggin and α-sFRP2 (together and with mitogens), as well as whether any new retinal neurogenesis ensues.

Disclosures: K.N. Grise: None. **L. Balenci:** None. **D. van der Kooy:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.07/C10

Topic: A.08. Transplantation and Regeneration

Title: DCLKs: New regulators of growth cone regeneration

Authors: *H. NAWABI[1], S. BELIN[1], R. CARTONI[1], C. WANG[1], A. LATREMOLIERE[1], X. WANG[1], C. WOOLF[1], C. V. GABEL[2], J. STEEN[1], Z. HE[1]
[1]NEUROLOGY, BOSTON CHILDRENS HOSPITAL HARVARD MEDICAL SCHOOL, BOSTON, MA; [2]Physiol. and Biophysics, Boston Univ. Sch. of Med., BOSTON, MA

Abstract: Axon regeneration in the adult central nervous system is extremely limited and the underlying mechanisms are still poorly understood. Transforming the stump of an injured axon into a motile growth cone structure has been implicated as a key rate-limiting step in axon regeneration. Using a comparative proteomic approach on FACS-purified RGCs, we found that the abundance of DCLK1/2 (doublecortin like kinase 1/2) is dramatically reduced in injured retinal ganglion cells (RGCs). DCLK1/2 share structure similarities with DCX (doublecortin), which is involved in neuronal migration, but their functional roles in mature neurons are not defined. Our further studies suggest a critical role of DCLKs in the growth cone reformation during optic nerve axon regeneration. First, we verified that the expression of DCLK1/2 decreases dramatically after injury. Second, when overexpressed in WT animals, DCLK2 induced a limited yet significant regeneration. Yet, in a PTEN deleted background, DCLK2 expression resulted in dramatically increased axon regeneration, in particular at the initiation stage of optic nerve regeneration. Third, our time lapse imaging studies on retinal explants implicate DCLK2 as being crucial to growth cone regeneration. Our current studies involve analyzing the phenotypes of growth cone regeneration in mice with the DCLK1/2 double knockout. We are also dissecting the molecular mechanisms by which DCLK2 executes its effects on growth cone formation and other stages of axon regeneration. Together, our results may reveal the key molecular players in the growth cone regeneration, which should be important for designing therapeutic strategies of promoting axon regeneration after injury.

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Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Title: Translational profiling of retinal ganglion cells in response to optic nerve injury in post-metamorphic Xenopus laevis

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Abstract: Mammals lose the ability to regrow the injured optic nerve shortly before birth, whereas the South African-clawed frog Xenopus laevis retains this regenerative capacity throughout development. To study how Xenopus laevis accomplish this, we seek to generate a translational profile for the regrowth of retinal ganglion cell (RGC) axons following injury. As a means of isolating RGC mRNAs from a heterogeneous population of retinal cells, we generated transgenic Xenopus laevis frog lines that express enhanced green fluorescent protein (EGFP) in the ribosomes of only RGCs. We crushed the optic nerves of juvenile Xenopus using forceps, collected tissue samples 0, 1, 3, 7, and 11 days post-surgery, and used translating ribosome affinity purification (TRAP) to isolate mRNAs being actively translated by ribosomes expressing EGFP. Here we present initial results showing differential gene expression patterns for ~16,000 genes sequenced using RNA-Seq, as well as subsets of RGC-enriched genes. These data indicate unexpected broad temporal patterns of expression: primarily up-regulation by Day 3, followed by widespread down-regulation in Days 7 and 11. Using quantitative PCR (qPCR), we validated RNA-Seq results for a subset of 14 genes with distinct expression patterns. Finally, we present results of clustering analysis to determine novel gene subsets that likely play a role in the

regeneration process. Molecules or signaling pathways discovered to facilitate the regeneration process may have potential for translation into clinical practice.

Disclosures: **D. Heinen:** None. **N. Marsh-Armstrong:** None. **F.L. Watson:** None. **D. Rhoades:** None. **A.H. Watson:** None. **B. Misaghi:** None. **C. Ives:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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The DOD

Title: The krüppel-like factor gene targeting dusp14 regulates axon growth and regeneration

Authors: ***Y. WANG**[1], K. IWAO[2], A. APARA[2], D. L. MOORE[2], M. BLACKMORE[3], N. J. KUNZEVITZKY[1], J. L. GOLDBERG[1]
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Abstract: Retinal ganglion cells (RGCs) and other central nervous system (CNS) neurons in adult mammals are unable to regenerate their axons after injury. Krüppel-like transcription factor (KLF) family members regulate intrinsic axon growth ability of CNS neurons during development, but downstream mechanisms have been elusive. By screening genes regulated by KLFs in RGCs, we identify Dual specificity phosphatase 14 (Dusp14) as a key player limiting axon growth and regenerative ability downstream of KLFs' ability to regulate axon growth in RCGs. We demonstrate Dusp14 expression increases in parallel to KLF9 during RGC development, and is induced by KLF9 in RGCs *in vitro*. The Dusp14 mediates neurite growth suppression *in vitro* in a phosphatase-dependent fashion, and the KLF9-Dusp14 pathway inhibits

activation of mitogen-activated protein kinases (MAPK), critical to neurotrophic signaling of RGC axon elongation. Decreasing Dusp14 expression or function in RGCs rescues KLF9-induced neurite outgrowth suppression *in vitro* and optic nerve regeneration after trauma *in vivo*. Thus, developing strategies to modulate this KLF-Dusp14 pathway to enhance MAPK signaling may further promote axon regeneration after CNS injury.

Disclosures: Y. Wang: None. **K. Iwao:** None. **A. Apara:** None. **D.L. Moore:** None. **M. Blackmore:** None. **N.J. Kunzevitzky:** None. **J.L. Goldberg:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: NIH R21 NS081467

Title: Regeneration of serotonin axons in the neocortex of the adult mouse probed with *in vivo* two-photon imaging

Authors: Y. JIN, *D. J. LINDEN
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Abstract: Experiments using fixed tissue have suggested that the serotonin (5HT) neurons of the dorsal raphe have an unusual and atypical capacity for axonal regeneration. However, these fixed tissue experiments cannot capture the dynamics of axonal regeneration and cannot easily distinguish true regeneration from the compensatory sprouting of undamaged axons or transient serotonin depletion. To address these issues, we employed chronic *in vivo* two photon imaging of the somatosensory cortex in an Slc6A4 (serotonin transporter)-soluble EGFP BAC transgenic mouse in which the complete extent of the serotonin-containing neurons was labeled. Consistent with previous work using fixed rat tissue, we found that repeated systemic injection of p-chloro-amphetamine (PCA) produced immediate and profound degeneration of 5HT axons but no loss of the 5HT-poisitve cell bodies of origin in the dorsal raphe. In the weeks to follow, a slow recovery was observed that was dominated by axon regeneration. Most of regenerated axons became morphologically stable and survived till the final monitoring point (27 weeks). The regenerated serotonin axons entered the neocortex individually rather than bundles and did not follow blood vessels or spared axons. They appear to avoid the spared axons and each other to

fill the imaged cortical volume efficiently. The spared axons observed soon after PCA treatment almost entirely survived to the final monitoring point and exhibited only a small degree of sprouting. In contrast to PCA treatment, saline treatment didn't cause any degeneration, sprouting or rearrangement of 5HT axons over the ensuing 27 weeks. Based on these descriptive results, we believe that time-lapse imaging of regenerating 5HT axons in somatosensory cortex may provide an ideal test bed to develop therapeutic strategies to promote axon regeneration and hence the recovery of function following brain injury.

Disclosures: Y. Jin: None. **D.J. Linden:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: R01-EY020297

P30-EY022589

Title: The role of mitochondrial fission/fusion in CNS axon regeneration

Authors: *A. KREYMERMAN[1,2], Y. WANG[3], N. J. KUNZEVITZKY[4], M. B. STEKETEE[6], J. L. GOLDBERG[5]
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Abstract: Central nervous system (CNS) disease or injury is often accompanied by progressive axon degeneration, leading to lost sensory, motor, or cognitive abilities, with little to no regenerative response. In search of signaling factors to restore degenerated CNS axons, we identified a group of developmentally regulated transcription factors, the Krüppel-like transcription factors (KLFs), which differentially suppress or enhance hippocampal, corticospinal neuron, and retinal ganglion cell (RGC) axon growth. However, the downstream mechanisms by which KLFs regulate axon growth are unknown. Evidence suggests one downstream effector may be mitochondrial (Mt) fission/fusion dynamics. We recently showed that suppressing fission (increasing fusion) leads to a loss in axon growth inhibition by

chondroitin sulfate proteoglycans, supporting a hypothesis in which CNS axon growth and guidance is regulated by Mt fission-fusion dynamics. These data also suggest suppressing Mt fission is a potential therapeutic strategy for improving axon regeneration after CNS trauma or disease. To identify whether Mt fission/fusion mechanisms also underlie the axon suppressing/enhancing activity of KLFs, we are investigating the potential ability for KLFs to critically regulate Mt genes for axon growth. Pertinent to our previous findings, we found that axon growth-suppressing KLF9 increased and growth-promoting KLF7 decreased the genetic expression of mitochondrial fission process 1,18 kDa (MTP18), a positive regulator of Mt fission, supporting the hypothesis that increased fission is inhibitory for axon growth in CNS neurons. Furthermore, our recent data analyzing exome sequencing of familial axonopathies also pointed to a disease association with a number of mitochondrial proteins thought to act on fission/fusion dynamics, including MTP18. Therefore, we hypothesize that KLF7/9-mediated regulation of the mitochondrial fission enhancer MTP18 regulates intrinsic axon growth ability in CNS neurons. To address this hypothesis, we will express/knockdown MTP18 in combination with or independent of KLF7/9 expression/knockdown in RGCs both *in vitro* and *in vivo*, identifying the neuronal role of MTP18 in regulating Mt fission/fusion dynamics, CNS axon growth and guidance, and KLF7/9-mediated axon regeneration. The overall goal is to improve our understanding of how Mt fission/fusion regulates axon regeneration and identify strategies for restoring axon growth after CNS injury or disease.

Disclosures: A. Kreymerman: None. **Y. Wang:** None. **N.J. Kunzevitzky:** None. **M.B. Steketee:** None. **J.L. Goldberg:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: Shriners Research Foundation grant SHC-85310

Title: Elevation of chemorepulsive axon guidance receptors UNC5 and Neogenin are involved in the “bad-regenerating” RS neuron death after spinal cord injury

Authors: *J. CHEN, C. LARAMORE, J. S. SHAHOUD, M. SHIFMAN
Temple Univ. Sch. of Med., Shriners Hosp. Pediatric Res. Ctr., Philadelphia, PA

Abstract: After complete spinal cord injury (SCI) in mammals injured axons are unable to regenerate. Both intrinsic and extrinsic factors associated with myelin and glial scar play important roles in limiting axonal regeneration. Many documents evidenced that a number of axon guidance molecules (eg. Netrin and RGM) are expressed in the adult CNS and their expression altered following injury. Growing axons receives signals from a plethora of both attractive and repulsive guidance cues that recognized by multiple receptors. Recently published studies suggest that axonal guidance molecule and their receptors play very important role not only during development but also at adult stage. We hypotheses that the inability of severed axons to undergo regeneration in the adult CNS is, at least in part, attributable to the presence of the very same molecules that were so important during development in establishing the axonal connections. In order to test the hypothesis that combination of multiple repulsive guidance molecules contribute to regeneration failure after SCI, we developed new sequential chromogenic triple-labeling *in situ* hybridization method, and investigated the Co-expression of Netrin receptors DCC, UNC5 and RGM receptor Neogenin mRNA in reticulospinal neurons of the larval lamprey brainstem. We found that in control animals, DCC, UNC5 and Neogenin are Co-expressed in both “good regenerating” and “bad regenerating” RS neurons. After SCI, neither Neogenin nor DCC or UNC5 mRNAs is expressed in “good regenerating” neurons. On the contrary, in the “bad regenerating” neurons, the expression of chemoattractive receptor DCC decreased, while the expression of chemorepulsive receptors Neogenin and/or UNC5 prominently increased from 1 month till two month after spinal cord transection. Further, in combination with FLICA staining and Dextran-retrograde labeling methods, we found these RS neurons with elevated expression of Neogenin and/or UNC-5 are going to undergo apoptosis and hardly to regenerate afterwards. While after injection of Neogenin antisense Morpholino oligos, some type of “bad regenerating” RS neurons can survive through 4months after SCI. Our data indicated chemorepulsive effects of Netrins and RGM may inhibit regeneration in those neurons and we are proposing the hypothesis that selective inhibition of chemorepulsive receptors (e.g. Neogenin and UNC5) will change balance between repulsive and attractive signaling, enhancing regenerative potential “bad regenerating” neurons and converting them into “good regenerating” neurons.

Disclosures: **J. Chen:** None. **C. Laramore:** None. **J.S. Shahoud:** None. **M. Shifman:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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NIH R15 NS081686

University of Puerto Rico

Title: Temporal and spatial analysis of neuronal fiber subpopulations in the regenerating radial nerve cord of the sea cucumber Holothuria glaberrima

Authors: *C. I. LOPEZ, L. VÁZQUEZ-FIGUEROA, D. CRESPO-VÉLEZ, J. E. GARCÍA-ARRARÁS
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Abstract: Regeneration mechanisms within the central nervous system (CNS) have yet to be fully unraveled. To understand these mechanisms, our lab studies the regenerating process in the CNS of the sea cucumber Holothuria glaberrima. We have previously shown that three commercial antibodies (anti-Pax6, anti-Nurr1, and anti-Phosphohistone H3) identify distinct fiber subpopulations within H. glaberrima CNS. In this study, our objective was to characterize the temporal and spatial regeneration patterns of these distinct subpopulations following transection of the holothurian CNS. To do this, one of the five radial nerve cords (RNC) of the sea cucumber wastransected with a scalpel and left to recover. Animals were sacrificed at different days post-injury (dpi): 2, 6, 12, 21, and 28 dpi. Using immunohistochemistry, the fiber subpopulations were labeled with anti-Pax6, anti-Nurr1, and anti-PH3 and analyzed. At 2 dpi, anti-Pax6 showed high expression in the cut ends of the RNC at the injury site. Anti-PH3 expression showed little changes during the first two weeks of regeneration, but appeared to be more prominent at the injury site in 21 dpi animals. Anti-Nurr1 labeled a distinct fiber population in the RNC whose expression appears to decrease at 21 dpi. The results suggest that the fiber population differs in their regeneration profile. These results provide insight into the spatial and temporal patterns of CNS regeneration that might help understand the regenerative capacities of these organisms.

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Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: NIH Grant R01NS064288

Title: MicroRNA-26a regulates mammalian axon regeneration by inducing GSK3β expression

Authors: ***J. JIANG**[1,2], C. LIU[1], X. SAIJILAFU[1], B. ZHANG[1], Z. JIAO[1], Y. HU[1], M. ZHANG[1], Y. GUO[1], J. YUAN[1], F. ZHOU[1]
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Abstract: MicroRNAs are key negatively epigenetic regulators of gene expression. But we know very little about the roles of MicroRNAs in axon growth and regeneration in the mammalian peripheral nervous system (PNS). Here we observed that MiR-26a regulates mammalian axon regeneration in the PNS. GSK3β is the functional target of miR-26a. The MiR-26a inhibitor decreases axon regeneration ability by directly increasing the endogenous expression level of GSK3β. Importantly, we provide the first evidence that MiR-26a and GSK3β modulate axon regeneration ability *in vivo*. Furthermore, we show that MiR-26a-GSK3β signal is conveyed by the induction of a transcription factor Smad1 and that overexpression of Smad1 rescues axon regeneration ability mediated by miR-26a inhibitor *in vitro*. Together, these results suggest MiR-26a-GSK3β-Smad1 signaling as a central module for controlling axon regeneration ability in the mammalian peripheral nervous system.

Disclosures: J. Jiang: None. **C. Liu:** None. **X. Saijilafu:** None. **B. Zhang:** None. **Z. Jiao:** None. **Y. Hu:** None. **M. Zhang:** None. **Y. Guo:** None. **J. Yuan:** None. **F. Zhou:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Title: Protein-protein interactions involved in the transcriptional control of intrinsic axon growth ability in retinal ganglion cells

Authors: *A. APARA[1], Y. WANG[2], A. TRILLO[4], K. IWAO[4], M. BLACKMORE[5], J. GOLDBERG[3]
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Abstract: Neurons in the adult mammalian central nervous system (CNS) decrease in intrinsic axon growth capacity during development in concert with changes in Krüppel-like transcription factors (KLFs), which regulate axon growth capacity in CNS neurons including retinal ganglion cells (RGCs). Here we find that knockdown of KLF9, an axon growth suppressor normally upregulated 250-fold in RGC development, promotes long-distance optic nerve regeneration *in vivo*. We identify a novel binding partner, MAPK10/JNK3 kinase, and find JNK3 is critical for KLF9's axon growth suppressive activity. Interfering with a JNK3-binding domain (JBD), or mutating two newly discovered serine phosphorylation acceptor sites, Ser106/Ser110, effectively abolished KLF9's neurite growth suppression *in vitro* and promoted axon regeneration *in vivo*. These findings demonstrate a novel, physiologic role for the interaction of KLF9 and JNK3 in regenerative failure in the optic nerve and suggest new therapeutic strategies to promote axon regeneration in the adult CNS.

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Poster

399. Intrinsic Mechanisms of CNS Regeneration

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NEI

Department of Defense

Title: Evaluating the role and effect of specific neuron-intrinsic pathways that mediate collateral axonal sprouting after CNS injury

Authors: *D. LEE, X. LUO, B. YUNGHER, J. LEE, K. PARK
Univ. of Miami Miller School/ Miami Project, Miami, FL

Abstract: Following injury, axonal remodeling in the form of collateral sprouting of spared axons that compensate for lost circuits represents a key mechanism by which behavioral functions are recovered. For instance, in the case of spinal cord injury (SCI), clinical lesions are often functionally incomplete, and thus compensatory sprouting of uninjured axons represents an important mechanism for functional recovery. Such axonal re-growth is robust after injury in young postnatal animals but diminishes with ageing. Using conditional knockout mouse and RNA interference, here we perform loss of functions studies to define the role of mTOR, STAT3, Dicer and MEK1/2 in neonatal or young adult neurons in regulating spontaneous collateral sprouting of corticospinal tract (CST) axons after pyramidotomy. Our findings demonstrate that mTOR is dispensable for the robust spontaneous sprouting of CST axons in immature mice. In contrast, moderate spontaneous axonal sprouting or induced-sprouting seen under different conditions in adult mice (i.e. PTEN deletion or inactivation of chondroitin proteoglycans) are reduced upon mTOR inhibition. In addition, we find that co-inactivation of PTEN and CSPGs leads to striking increase in axonal sprouting, and enhances functional recovery compared to single treatments. We also present our primary findings on the effects of depleting STAT3, Dicer or MEK1/2 in triggering spontaneous axonal sprouting in immature or young adult mice.

Disclosures: **D. Lee:** None. **X. Luo:** None. **B. Yungher:** None. **J. Lee:** None. **K. Park:** None.

Poster

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Support: Paralyzed Veterans of America 2981

Craig H. Neilsen Foundation 190270

Title: DRGs haploinsufficient for the mRNA binding protein IMP1 show increased regenerative properties and alterations in the PTEN/mTOR pathway

Authors: *A. L. HAWTHORNE[1], P. L. PRICE[1], J. L. TWISS[2], G. J. BASSELL[1]
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Abstract: Spinal cord injury (SCI) results in loss of function due to the failure of axon regeneration through an inhibitory environment in the CNS. Promoting axonal growth can be accomplished by relieving inhibition from the extracellular environment or through enhancing the neuron's intrinsic growth potential. Research from our lab and others has shown that local protein synthesis in axons plays a vital role in neuronal development and axon regeneration in the PNS. A major gap in our current understanding is that very little is known about the molecular mechanism and regulation of mRNA translation and local protein synthesis in adult CNS neurons following SCI. An increased understanding of how SCI effects local translation in regenerating axons is expected to lead to the identification of novel therapeutic strategies. Our recent work has shown that haploinsufficiency of the mRNA binding protein, IMP1/ZBP1/IGF2BP1, results in axon outgrowth impairments of adult naïve dorsal root ganglion (DRG) neurons in culture on a growth-promoting laminin substrate and in a peripheral nerve injury model *in vivo* (Donnelly *et al*., 2011). However, the role of IMP1 in axon growth in the more inhibitory environment of a CNS lesion is unclear. Here we have investigated the role of the IMP1 in axon regeneration *in vitro* on permissive and inhibitory substrates, as well as in response to a preconditioning lesion. When grown on more challenging substrates containing either low levels of laminin or an inhibitory aggrecan/laminin mix, the difference in axon outgrowth between WT and *Imp1*$^{+/-}$ DRGs was not significant. Conditioning did stimulate neurite outgrowth in both genotypes. However, when tested in an *in vitro* model of the inhibitory CNS lesion environment, the spot assay, unconditioned *Imp1*$^{+/-}$ DRGs surprisingly exhibited a marked increase in axon crossing comparable to conditioned WT DRGs. Conditioning did not further augment *Imp1*$^{+/-}$ axon crossing, as was observed with WT DRGs. We further observed that growth cones from *Imp1*$^{+/-}$ DRGs exhibited an increase in markers of translational activity, including phosphorylated ribosomal S6 protein, which is down stream in the PTEN/mTOR pathway. IMP1 associated with *β-actin* and *Pten* mRNA by co-immunoprecipitation. *Imp1*$^{+/-}$ DRGs have significantly less *Pten* and *β-actin* mRNAs than WT in axonal fractions as compared to γ-actin. We hypothesize that deficiency of IMP1 may impair axon growth in a permissive environment, yet allow for axon growth on an inhibitory substrate due to elevated mTOR

pathway activation. These results from *in vitro* paradigms will motivate *in vivo* experiments in spinal cord injured mice to possibly enhance axon outgrowth after injury.

Disclosures: **A.L. Hawthorne:** None. **P.L. Price:** None. **J.L. Twiss:** None. **G.J. Bassell:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Ziegler Foundation

Title: Translational analysis of retinal ganglion cells undergoing axon regeneration

Authors: ***E. BRAY**, K. LYAPICHEV, X. LUO, J. LEE, K. PARK
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Abstract: Neurons of the central nervous system fail to mount a strong regenerative response to axotomy. Although advances have been made in understanding how to overcome barriers to regeneration through modulation of cell intrinsic and extrinsic factors, the spatial and temporal domains of the induced regeneration remain limited. By utilizing a cell type specific RNA immunoprecipitation method coupled with RNA sequencing technology we wish to investigate the pool of RNA undergoing active translation in retinal ganglion cells (RGCs) induced to regenerate. Conventional methods for purifying a cell type from a complex tissue have multiple shortcomings. Fluorescence-activated cell sorting and immunopanning require tissue dissociation and hours of prep during which viable cells must be maintained, potentially resulting in major changes to the translational pool of RNA. Laser capture microdissection can be time intensive and presents difficulties in maintaining high quality RNA. Here we wish to utilize the Ribotag method to obtain cell type specific RNA in order to better understand the mechanisms by which RGCs regenerate. Viral delivery of Cre-recombinase to RGCs is used to induce expression of hemagglutinin tagged ribosomes for use as an IP target. The resulting mRNA pulled down by

this method will undergo RNA sequencing and computational analysis. Following optic nerve crush the RNA undergoing translation in untreated RGCs and those induced to regenerate will be compared. By utilizing this method we plan to establish a specific view of the *in vivo* translational network of RGCs in order to find new therapeutic targets that promote regeneration.

Disclosures: **E. Bray:** None. **K. Lyapichev:** None. **X. Luo:** None. **J. Lee:** None. **K. Park:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Title: Zinc is a critical regulator of optic nerve regeneration

Authors: ***Y. LI**[1,2], L. ANDEREGGEN[1], K. OMURA[1], B. ERDOGAN[1], M. S. ASDOURIAN[1], C. SHROCK[1], H.-Y. GILBERT[1], Y. YIN[1], U.-P. APFEL[3], S. J. LIPPARD[3], P. A. ROSENBERG[1], L. I. BENOWITZ[1]
[1]Children's Hosp. Boston and Harvard Med. Sch., Boston, MA; [2]State Key Lab. of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen Univ., Guangzhou, China; [3]Dept. of Chem., MIT, Cambridge, MA

Abstract: Like other pathways in the mature central nervous system (CNS), the optic nerve cannot regenerate if injured, resulting in lifelong losses in vision. Recent studies have achieved a moderate level of axon regeneration in the injured optic nerve by combining three treatments that synergistically activate the intrinsic growth state of retinal ganglion cells (RGCs), the projection neurons of the eye. Nonetheless, even under optimal conditions, more than 50% of RGCs die after optic nerve injury and most of the surviving RGCs fail to regenerate axons. These observations point to the existence of additional major suppressors of RGC survival and axon

regeneration. Using autometallography (AMG) or the fluorescent Zn2+ sensor ZinPyr1 (courtesy Robert Radford, MIT), we found that levels of free Zn2+ increased in the inner plexiform layer (IPL) of the retina within an hour of optic nerve injury, whereas levels within RGCs themselves increased more slowly. The IPL contains synaptic inputs onto the dendrites of RGCs from retinal interneurons (amacrine cells and bipolar cells). The Zn2+ transporter ZnT3 is highly expressed in the IPL, suggesting that Zn2+ becomes sequestered in presynaptic vesicles after injury. Chelating Zn2+ using either TPEN or the highly selective chelator ZX1 eliminated the Zn2+ signal in the IPL and led to enduring survival of RGCs as well as considerable axon regeneration. These effects were lost when the chelators were saturated with Zn2+, suggesting that the effects of the chelators are mediated through binding of free Zn2+. Combining Zn2+ chelation with other pro-regenerative treatments enabled some RGCs to regenerate axons the full length of the optic nerve in just 2 weeks. These studies indicate that Zn2+ is an endogenous suppressor of axon regeneration, and they suggest that Zn2+ chelators may be valuable in promoting recovery after various forms of traumatic or neurodegenerative CNS damage.

Disclosures: **Y. Li:** None. **L. Andereggen:** None. **K. Omura:** None. **B. Erdogan:** None. **M.S. Asdourian:** None. **C. Shrock:** None. **H. Gilbert:** None. **Y. Yin:** None. **U. Apfel:** None. **S.J. Lippard:** None. **P.A. Rosenberg:** None. **L.I. Benowitz:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Title: Sustained neurogenesis-an instrument for a successful regeneration in adulthood

Authors: *J. NINKOVIC, J. BARBOSA, R. DI GIAIMO, M. IRMLER, D. TRÜMBACH, J. BECKERS, M. GÖTZ
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Abstract: The regenerative capacity in the CNS differs greatly between different vertebrate species. Importantly, wide-spread, life-long neurogenesis is often associated with the remarkable regenerative potential. Indeed, the neural progenitors from the neurogenic zones of zebrafish telencephalon engage in the repair process without impairment of the constitutive neurogenesis. Therefore, we followed the response of a different type of progenitors to the brain injury at the single cell level *in vivo*. We first could show that only fast dividing progenitors, labelled with the

retroviral vectors, immediately react to the injury, leave the neurogenic zone and migrate towards the injury site. In contrast to fast dividing progenitors, the injury induced proliferation of neural stem cells, the radial glia-like cells, is delayed and coincident with the full tissue restoration. To assess the role of radial glia-like cells in the regeneration process, we continuously followed them for more than 1 month using the two-photon live imaging. Our data suggest the activation of previously quiescent radial glia-like cells in response to the injury, but with the different mode of division. The radial glia in the intact telencephalon show either asymmetric or self-renewing symmetric divisions, while the symmetric non-gliogenic division becomes the predominant type of symmetric division in response to injury. This change in the division mode leads to the depletion of some activated radial glia-like cells and production of the transit amplifying progenitor population that is used up in the neuronal regeneration process, but necessary for the constitutive neurogenesis. We could, further, identify the injury-induced activation of several pathways in progenitor cells. The activation of these pathways, using the small molecules, elicited both proliferation of progenitor cells and gene expression comparable to the injury. Moreover, the activation of these pathways in the injured mouse cortex increased the de-differentiation of the reactive astrocytes and increased their neurosphere-forming capacity. Taken together, our data describe for the first time the reaction of the endogenous progenitors to the injury at the single cell level and identify evolutionary conserved molecular pathways, involved in the initiation of the repair process by the endogenous progenitors in the vertebrate brain.

Disclosures: **J. Ninkovic:** None. **J. Barbosa:** None. **R. Di Giaimo:** None. **M. Irmler:** None. **D. Trümbach:** None. **J. Beckers:** None. **M. Götz:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.21/C24

Topic: A.08. Transplantation and Regeneration

Support: -NIH Annual Intramural Research Report Number: 1ZIAHL006021-05

Title: Differential binding of receptor protein tyrosine phosphatase type iia family (rptpσ/rptpδ/lar) to glycosaminoglycans

Authors: *A. A. MORGAN[1], Y. KATAGIRI[2], P. YU[2], N. J. BANGAYAN[2], R. JUNKA[2], H. M. GELLER[2]
[2]Natl. Heart, Lung, and Blood Inst., [1]NIH, BETHESDA, MD

Abstract: Abstract Body: Type IIa Receptor protein tyrosine phosphatases (RPTPs) have been shown to modulate neural regeneration and development. All members of this family (RPTP, RPTP, and LAR) have a cell adhesion molecule-like extracellular domain that includes three N-terminal Ig domains and four to nine fibronectin type III (FNIII) domains, as well as tandem intracellular tyrosine phosphatase domains. Along with protein tyrosine kinases, protein tyrosine phosphatases are crucial to the regulation of cellular protein tyrosine phosphorylation. This family of leukocyte common antigen-related receptors has been shown to influence axonal outgrowth and guidance during neural development. Compelling evidence suggests that both heparan sulfate (HS) and chondroitin sulfate (CS) are the ligands for RPTP and LAR, and the Lys-loop located in the first Ig domain is responsible for the ligand binding. However, the functional outputs of HS and CS are promotion and inhibition of axonal growth, respectively. The opposite effects of HS and CS glycosaminoglycans (GAGs) on axonal growth were attributed to the differential oligomeric state of RPTP because binding to HS, but not to CS, induces clustering of the extracellular region of RPTP. Here we show that all RPTP type IIa members display high affinity binding for heparin. Both CS-E and dermatan sulfate (DS) have similar dissociation constants within the typical nanomolar range, however, there is considerable variation of binding sites on GAGs among RPTPs. We also demonstrate differential contribution of FNIII domains required for high affinity binding to GAGs. In particular, PTP binding to heparin was not completely abolished by the disruption of the Lys-loop. This along with a decrease in binding with deletion of the fourth FN domain leads us to hypothesize that there is a greater contribution of the FN domain in binding to glycosaminoglycans that previously believed. Preliminary data shows that the fourth fibronectin repeat is able to bind to heparin without contribution of the three Ig domains. Furthermore, only RPTP is clustered upon binding to both heparin and CS-E, but not DS. Despite 66% overall shared amino acid identity amongst RPTPs, we conclude there exist discrete binding sites on RPTPs that determine binding to GAGs. Our data indicate that clustering of the receptor may not explain the distinct biological functions of HS and CS.

Disclosures: A.A. Morgan: None. **Y. Katagiri:** None. **P. Yu:** None. **N.J. Bangayan:** None. **R. Junka:** None. **H.M. Geller:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.22/C25

Topic: A.05. Axon and Dendrite Development

Title: AKT regulates Id2 for axonal regeneration in CNS

Authors: *H. KO[1], J. CHO[2], K.-H. LEE[2], J.-Y. AHN[2]
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Abstract: Id2 is a negative regulator of basic helix-loop-helix (bHLH) transcription factors. Id2 dimerizes and prevents bHLH from binding to E box DNA response elements that modulate neurogenetic transcription during development. Recent report showed that Id2 may be a novel target for enhancing sensory axonal regeneration following injuries to the adult spinal cord. However, how Id2 exerts axonal regeneration is unclear. In this study we demonstrate that Id2 interacts with AKT and is phosphorylated by Akt. Id2 phosphorylation is required for controlling Id2 protein levels and the interaction between Id2 and E3 ligase Cdh1. Moreover, AKT regulates the interaction of Id2 with transcription factors of E47. E47 belongs to the bHLH (basic helix-loop-helix) family of transcription factors and complex with NeuroD. NeuroD/E47 promotes the activation of myelin gene expression and inhibition of axon growth. Therefore, we suggest that regulation of Id2 by AKT has important role of axonal regeneration.

Disclosures: **H. Ko:** None. **J. Cho:** None. **K. Lee:** None. **J. Ahn:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 399.23/C26

Topic: A.05. Axon and Dendrite Development

Support: R01NS064288

Craig H. Neilsen

Title: H3k27 methylation balance regulates axon regeneration

Authors: ***C. LIU**[1,1], R.-Y. WANG[3], J.-J. JIANG[3], Z.-X. JIAO[1], S. XXX[1], B.-Y. ZHANG[1], S. ZHANG[1], M. ZHANG[1], Y.-W. HU[1], F. ZHOU[1,2]
[1]Dept. of Orthopaedic Surgery, [2]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Orthopaedic Surgery, Johns Hopkins Univ., Baltimore, MD

Abstract: The devastating and permanent disabilities after spinal cord and other types of mammalian central nervous system (CNS) injury is caused by the failure of injured axons to regenerate and to re-build the functional circuits. Neurons in the mammalian CNS lose their intrinsic ability to support neural regeneration. Regulated gene expression is one of the intrinsic ability determinations of neurons to extend axons. Methylation (mono-, di-, and tri-methylation) on histone H3 lysine (K) 27 induces transcriptional repression, and thereby participates in controlling gene expression patterns. Methyltransferase EZH2/EZH1 and de-methyltransferase JMJD3/UTX play essential roles in the epigenetic maintenance of the H3K27me3 repressive chromatin mark, however, their roles in mammalian axon regeneration are not well explored. Here we report that H3K27me3 methylation balance mediated by EZH2/EZH1 and JMJD3/UTX is a novel modulator of axon regeneration. H3K27me3 expression level is increased after axotomy in DRG neurons, and the expression levels of EZH2, JMJD3/UTX are also changed. We provide the first evidence that EZH2 knockdown impaired and JMJD3 knockdown promoted mammalian axon regeneration *in vivo*. Moreover, we found that KLF4 acts as a transcriptional downstream target of H3K27me3 to mediate axon regeneration in adult sensory neurons in response to peripheral nerve injury. Therefore, H3K27 methylation balance mediated by EZH2/JMJD3/UTX provides a novel mechanism for controlling intrinsic axon regeneration ability in adult dorsal sensory neurons

Disclosures: C. Liu: None. **R. Wang:** None. **J. jiang:** None. **Z. Jiao:** None. **S. xxx:** None. **B. zhang:** None. **S. zhang:** None. **M. zhang:** None. **Y. Hu:** None. **F. Zhou:** None.

Poster

399. Intrinsic Mechanisms of CNS Regeneration

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: A.05. Axon and Dendrite Development

Support: National Natural Science Foundation of China 31200826

National Basic Research Program of China 973 Program 2011CB504402

Title: Celsr3 is required in hippocampal projection neurons for network wiring and spatial learning

Authors: *Y. HUANG, J. FENG, Y. DING, K.-F. SO, L. ZHOU, Y. QU
GHM Inst. of CNS Regeneration, Guangdong, China

Abstract: The atypical cadherin Celsr3, which belongs to the core planar cell polarity group, orchestrates axonal guidance and network wiring. Previous work using regional inactivation of Celsr3 in forebrain showed that Celsr3 is widely involved in hippocampal maturation and connectivity. However, inactivation in the whole forebrain does not provide sufficient specificity to address the function of Celsr3 in detail. Here, we studied the Celsr3|Emx1 mouse mutant model, in which Celsr3 is selectively inactivated in hippocampal projection neurons, but not in entorhinal cortex, basal ganglia and interneurons. In that mutant, the hippocampal cytoarchitecture was almost normal. Inactivation of Celsr3 in projection neurons perturbed intrinsic hippocampal wiring and efferent fiber projections, whereas afferent fibers from entorhinal cortex were unaffected. In addition, it resulted in incomplete maturation of pyramidal cell dendrites and synaptogenesis, and increased adult neurogenesis in the dentate gyrus. Consistent with these wiring defects, Celsr3|Emx1 mutant mice showed impaired learning and memory, and were less anxiety-prone than control mice. Thus, Celsr3|Emx1 mutant mice provide a simple way to study the consequences of defective hippocampal wiring in absence of drastic structural anomalies.

Disclosures: **Y. Huang:** None. **J. Feng:** None. **Y. Ding:** None. **K. So:** None. **L. Zhou:** None. **Y. qu:** None.

Poster

400. Invertebrate Transmission

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Program#/Poster#: 400.01/C28

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant RR03051

NIH Grant MD007600

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NSF Grant DBI-0115825

Title: Localization of BgNPY-like immunoreativity in the nervous system of Biomphalaria glabrata, an intermediate host for schistosomiasis

Authors: *S. ROLÓN-MARTÍNEZ[1,2], N. DELGADO-RIVERA[1,2], G. TORRES[1,2], L. O. VAASJO[1,2], E. RIVERA[1,2], M. W. MILLER[1,2]
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Abstract: Over 200 million people worldwide live at risk of the parasitic disease schistosomiasis, or "snail fever". The digenetic trematode worm species *Schistosoma mansoni* that causes the most widespread form of intestinal schistosomiasis, employs the freshwater snail *Biomphalaria glabrata* as its primary intermediate host. Previous investigations in other schistosome-snail systems (*Trichobilharzia ocellata – Lymnaea stagnalis*) showed that neuropeptide Y (NPY) gene expression was increased during the "shedding" stage of snail infection, when a high production and release of parasites (cercariae) requires considerable redirection of host energy resources (Hoek et al. 1997; de Jong Brink et al. 1999). A recently submitted mRNA sequence was reported to encode a *B. glabrata* NPY prepropeptide (GenBank Accession No.: JX013957). For this investigation, affinity purified polyclonal antibodies (rabbit) were generated against part (20 amino acids) of the predicted NPY neuropeptide (BgNPY) deduced from the mRNA sequence. BgNPY-like immunoreactive neurons (20 – 30 um diameter) were present on the dorsal and ventral surfaces of most central ganglia; buccal ganglion (B. g.; dorsal: 9 ± 2, ventral: 3 ± 1), cerebral ganglion (Cer. g.; dorsal: 17 ± 2, ventral: 19 ± 7), pedal ganglia (Pd. g.; dorsal: 20 ± 6, ventral: 16 ± 8), and pleural ganglia (Pl. g.; dorsal: 4 ± 1, ventral: 2 ± 1). Larger (40 - 50 um diameter) BgNPY-li neurons in the left parietal ganglion (Pa. g.; dorsal: 14 ± 5, ventral: 7 ± 4), and visceral ganglion (V. g.; dorsal: 23 ± 3, ventral: 12 ± 6) had prominent axons oriented toward the parietal-visceral connective. Areas of the sheath surrounding the ganglia and specific nerves, such as the tentacular nerve and medial lip nerve, were rich in BgNPY-like fibers, suggesting that this peptide could be released in a neurohaemal fashion. BgNPY-like staining was also found in the statocysts. These results suggest that BgNPY could be involved in behaviors such as food intake and reproduction, and are consistent with a role of this neuropeptide in the redirection of energy resources during the stage of cercarial shedding in the *Schistosoma mansoni* - *Biomphalaria* host-parasite system.

Disclosures: S. Rolón-Martínez: None. **N. Delgado-Rivera:** None. **G. Torres:** None. **L.O. Vaasjo:** None. **E. Rivera:** None. **M.W. Miller:** None.

Poster

400. Invertebrate Transmission

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 400.02/C29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH

NSF

NASA

Title: Genomic portrait of a synapse: Enormous variability and unexpected complexity in the molecular complement

Authors: *A. B. KOHN[1], L. L. MOROZ[2,3]
[1]Universtiy of Florida Whitney Lab., Saint Augustine, FL; [2]Neurosci. and McKnight Brain Inst., Univ. of Florida, Gainesville, FL; [3]The Whitney laboratory for Marine Biosci., St Augustine, FL

Abstract: Advances of single-neuron genomics allowed us to obtain an unbiased view of the genomic organization of both neurons and synapses within identified neural circuits. Here, we took advantage of the sequenced *Aplysia* genome and well-developed transcriptome profiling from various neurons to characterize the complement of genes associated to synaptic functions and excitability. Specifically we targeted several identified components of defensive feeding circuits. Our genomic analysis of the *Aplysia* glutamatergic synapse revealed a significantly greater complexity compared to recently reported hippocampal synapses in rodents and the vertebrate synapse in general. In part, we detected higher diversity of the *Aplysia* ionotropic glutamate receptors (iGluRs, 23 compared to the number of human iGluRs at 18). We also determined there are greater number of predicted secretory molecules in pre- and postsynaptic neurons including novel types of prohormones. The overall complement of ion channels in *Aplysia* (197) is comparable to the human complement at 237 ion channels. However, in *Aplysia* we identified, a number of subfamilies significantly expanded in the molluscan lineage compared to all other animals. Second, we also revealed an enormous diversity and differential recruitments of various components controlling excitability and synaptic efficiency (both across and within the same classes of neurons and synapses). We hypothesize that the observed redundancy and variability can be considered as adaptive mechanisms controlling synaptic homeostasis and robustness of synaptic functions. The obtained data together with comparative genomic analysis across basal metazoans allowed us to reconstruct the early origins and parallel evolution of synaptic functions in animals.

Disclosures: **A.B. Kohn:** None. **L.L. Moroz:** None.

Poster

400. Invertebrate Transmission

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: GVSU S3 grant to AH

GVSU Presidential Grant to MM

Title: Use of an internal FLAG epitope for biochemical characterization and localization of histidine decarboxylase (HDC) in tissues of *Drosophila* melanogaster

Authors: *M. G. BURG[1], M. MIANECKI[2], A. HAGE[3], D. BURG[3]
[1]Biomed. Sciences/Cell and Mol. Biol., Grand Valley State Univ., ALLENDALE, MI; [2]Cell and Mol. Biol., [3]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Histamine (HA) is synthesized by the enzyme histidine decarboxylase (HDC) that is encoded by the *Hdc* gene in *Drosophila*. The analysis of *Hdc* mutants defective in HA synthesis and regulation of *Hdc* expression has, in the past, relied solely upon HA immunohistochemistry to assess HDC activity. Determining the location of the *Drosophila* HDC protein in various tissues and isolating HDC for biochemical studies has been hampered due to the inability to detect HDC immunohistochemically. Efforts in the past to label the HDC protein, including N- or C-terminal labeling using an epitope tag such as 6XHIS or FLAG, have been unsuccessful. This failure to label HDC is likely due to the fact that, as in other species, the protein is post-translationally modified by the removal of both the N- and C-terminus (ref.1), preventing the use of conventional N- and C-terminal epitope-labeling techniques to label the HDC protein. Several internal sites for an epitope label within the *Drosophila Hdc* gene were identified, one of which could accept an insertion of a small epitope label (6XHIS) without disrupting the HDC protein's function (ref. 2). Using this same approach, a FLAG epitope was placed into the same internal site of the *Hdc* gene structure, resulting in a FLAG-*Hdc* transgene. The FLAG-*Hdc* transgene was placed into a plasmid vector and transformed into *Drosophila* using standard techniques. Linkage of the FLAG-*Hdc* transgene was determined for each transformant line generated and selected transformant-bearing flies were then crossed into a mutant Hdc^{JK910} background lacking endogenous HDC activity. These transgenic flies were analyzed for a functional HDC protein by immunostaining with HA antibodies in a number of developmental stages. A similar procedure was used to detect the epitope-labeled FLAG-HDC protein using the M2 FLAG antibody. Results for the FLAG-HDC epitope immunodetection yielded a staining pattern very similar to

the HA immunostaining in the wildtype fly. Co-localization experiments for HA and FLAG-HDC have been completed in both the larval and adult CNS, indicating that FLAG expression co-localizes with HA. Immunoprecipitation experiments using the M2 FLAG antibody have identified a 52 kD protein specific to the FLAG-*Hdc* transformant, which we propose to be the size of the HDC monomer in *Drosophila*. These epitope tagged FLAG-*Hdc* transgenic flies can now be used to further our understanding of HDC biochemistry and cell biology *in vivo*. (1) Fleming and Wang (2000) Molec. Cell Biol. 20: 4932-4947. (2) Fair et al. (2012) 53rd *Drosophila* Research Conference.

Disclosures: **M.G. Burg:** None. **M. Mianecki:** None. **A. Hage:** None. **D. Burg:** None.

Poster

400. Invertebrate Transmission

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Program#/Poster#: 400.04/C31

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: A role for dopamine in peripheral sensory processing in the predatory sea-slug Pleurobranchaea

Authors: B. M. SCHAUB[1], B. L. KLUSAS[3], J. W. BROWN[4], D. I. VALLEJO[5], N. DELGADO[6], N. M. MADEIROS[6], S. R. MARTINEZ[6], E. BARRETO[6], M. W. MILLER[6], *R. GILLETTE[2]
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Abstract: The peripheral nerve networks of the chemotactile oral veil-tentacle complex and rhinophores of the predatory sea-slug *Pleurobranchaea californica* show extensive tyrosine hydroxylase immunoreactivity (TH-IR), consistent with dopaminergic innervation. TH-IR is also marked in ciliated, epithelial putative primary chemoreceptor cells, as well as in downstream somata of the associated peripheral ganglia. Some of these TH-IR somata project into the sensory Large Oral Veil and Tentacle Nerves (LOVN, TN), which supply the CNS with information critical to cost-benefit decisions serving foraging, defense, and reproduction (Brown et al., in preparation). We tested a potential role for dopamine in both food-seeking behavior and the underlying peripheral sensory physiology with sulpiride, a dopamine D2/D3 receptor antagonist. Appetitive responses were observed to application of raw shrimp on both sides of the oral veil-tentacle complex before and after unilateral topical application of sulpiride. Sulpiride

significantly increased latency to orient to and bite at food stimuli applied to the treated side ($p < 0.001$, Wilcoxon matched pairs test), while latencies did not significantly vary on the control side before and after treatment. Recordings of sensory activity from LOVN and TN were consistent with the above observations. Unilateral sulpiride application significantly attenuated neural responses to tactile and appetitive chemotactile stimuli in decerebrated preparations. Thus, behavioral and physiological results are consistent with a pivotal role for peripheral dopaminergic pathways in sensory transduction and/or downstream peripheral integration.

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Poster

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CSUN Research and Sponsored Projects

Title: Identifying serotonergic neurons in the brain of the cockroach Rhyparobia maderae implicated in the regulation of carbohydrate self-selection feeding behavior

Authors: *L. DANIELS, JR, R. W. COHEN
Biol., California State University, Northridge, Northridge, CA

Abstract: Cockroaches like the madeira cockroach *Rhyparobia maderae* possess the ability to self-select a balanced diet based on nutritional need. Given a choice between a carbohydrate diet and a protein diet, an *R. maderae* nymph will feed 80% on the carbohydrate cube and only 20% on the protein cube. Previous work in our lab has shown that various neurotransmitters function to regulate this self-selection feeding behavior. For example, it appears that octopamine regulates hunger, while dopamine controls satiety. In contrast, serotonin is known to regulate specific nutrient self-selection in many animals, including *R. maderae* nymphs. Precisely, serotonin release in the cockroach brain is correlated with carbohydrate consumption: high levels cause reduced feeding while lower levels increase carbohydrate feeding. In the present study, immunohistochemistry was utilized to determine which regions of the *R. maderae* brain are involved in the regulation of carbohydrate self-selection behavior by serotonin. In determining

the pattern of serotonin release in the brain of actively feeding *R. maderae*, large nymphs (n=10 per treatment group) were randomly selected and divided into five treatment groups. Prior to treatment, nymphs were starved for seven days and allowed only water as sustenance. After seven days, nymphs were placed into individual glass petri dishes that were used as feeding arenas. Each arena contained a diet block and water *ad libitum*. The experimental treatment groups included one group feeding on carbohydrate (sucrose) diet cubes for 1 hour, a second group feeding on sucrose for 2 hours, a third group feeding on casein (protein) for 1 hour, and a fourth group feeding on casein for 2 hours. Unfed, control groups were used to compare to the other feeding treatment groups. After 1-2 hours of feeding, the *R. maderae* nymphs were anesthetized with CO_2, and their brains were dissected out. All brains were fixed by incubating in 30% sucrose in 4% paraformaldehyde solution for three days. Brains were then sectioned with a cryostat at 25µm thickness and prepped for immunohistochemistry. Briefly, brain sections were incubated in 1% primary antibody (serotonin), normal goat serum and PBS for 2 hours. The sections were then incubated in secondary antibody (anti-rabbit), normal goat serum and PBS for one hour. Our results show a very high localization of serotonin in and around the mushroom bodies of cockroach brain. This finding corroborates our hypothesis that the insect mushroom body is involved in carbohydrate self-selection behavior.

Disclosures: L. Daniels: None. **R.W. Cohen:** None.

Poster

400. Invertebrate Transmission

Location: Halls A-C

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Topic: B.01. Neurotransmitters and Signaling Molecules

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Title: Histamine-like immunoreactivity in the central and peripheral nervous systems of Biomphalaria spp., intermediate host snails for schistosomiasis

Authors: M. R. HABIB[1,2], A. H. MOHAMED[3], G. Y. OSMAN[3], A. T. SHARAF EL-DIN[1], H. S. MOSSALEM[1], N. DELGADO[4,5], G. TORRES[4,5], S. ROLON-MARTINEZ[4,5], *M. W. MILLER[4,5], R. P. CROLL[2]
[1]Med. Malacology Lab., Theodor Bilharz Res. Inst., Giza, Egypt; [2]Physiol. & Biophysics, Dalhousie Univ., Halifax, NS, Canada; [3]Zoology Dept., Minufiya Univ., Shebin El-Kom, Egypt; [4]Inst. Neurobio., San Juan, PR; [5]Anat. & Neurobio., Univ. of Puerto Rico Med. Sci. Campus, San Juan, PR

Abstract: Snails of the genus *Biomphalaria* are intermediate hosts for the digenetic trematode parasite *Schistosoma mansoni*, which infects people living in tropical regions of Africa and the Americas. It has been proposed that the development of *S. mansoni* from the free-living miracidium to the parasitic mother sporocyst stage depends upon uptake of biogenic amines from the snail host (see Boyle et al. 2003; El-Shehabi and Ribeiro 2010). Previous studies have demonstrated the presence of histamine in *Schistosoma* (Ribiero et al. 2005) but no data are available concerning possible sources of histamine in the tissues of *Biomphalaria*. This investigation examined the localization of histamine-like immunoreactive (HA-Lir) material in the central nervous system (CNS) and peripheral tissues of both *B. alexandrina* (the snail host in Egypt) and *B. glabrata* (the snail host in the American tropics). Evidence indicates that HA is plentiful throughout the nervous system of the snail. Within the CNS, clusters of HA-Lir neurons were observed in the buccal, cerebral, pedal, left parietal, and visceral ganglia, suggesting involvement in a variety of neural functions. Histamine was also found in the hair cells of the statocyst indicating involvement in graviception. Finally, numerous peripheral cell bodies in the cephalic sensory organs and anterior margin of the foot, together with the heavy labeling of nerves connecting these regions with the CNS indicates that populations of chemo- and/or mechanoreceptor afferent cells also use HA as their transmitter. The patterns of staining for both species of *Biomphalaria* were similar to each other and to the staining observed in other pulmonates examined to date. The results thus indicate that the snail hosts have abundant sources of HA that could be exploited by larval schistosomes and suggest numerous host-parasite interactions involving this aminergic transmitter.

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Poster

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Program#/Poster#: 400.07/C34

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH

NFS

NASA

Title: Establishing and maintenance of neuronal identity in *Aplysia*: Tracing the origins of serotonergic and dopaminergic neurons in bilaterians

Authors: *E. C. DABE[1], A. B. KOHN[2], L. L. MOROZ[2]
[1]Univ. of Florida Whitney Lab., Saint Augustine, FL; [2]Univ. of Florida, Gainesville, FL

Abstract: *Aplysia californica* neurons are some of the largest somatic cells in the animal kingdom and contain enough genetic material per cell to allow for in depth transcriptome analysis. In order to understand the logic of gene regulation and gene expression changes underlying complex neuronal phenotypes, we performed RNA-seq on freshly isolated individual *Aplysia* neurons. By using this specific single-cell transcriptome approach, we were able to identify a subset of gene candidates involved in the maintenance of unique phenotypes of serotonergic and dopaminergic neurons such as transcription factors in the *ETS*, *Hox* and *Pitx* subfamilies. *In situ* hybridization was then performed to map the expression pattern of these candidate regulators in the *Aplysia* nervous system to identify and define cells with specific neurotransmitter phenotypes Computational approaches then identified potential transcription factor binding sites in the promoter regions of the genes encoded dopamine and serotonin synthetic pathways respectively. Together, our data on the regulation of neurotransmitter expression suggest both serotonergic and dopaminergic neuronal cell lineages arose from a common bilaterian ancestor. Supported by NIH, NSF and NASA

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Poster

400. Invertebrate Transmission

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GSU Brains & Behavior Seed Grant

NSERC

Title: Brain transcriptomes from six sea slug species provide insights into neural circuit evolution

Authors: A. SENATORE, *P. S. KATZ
Neurosci. Inst., Georgia State Univ., ATLANTA, GA

Abstract: Neuronal circuits in the clade Nudipleura (Mollusca, Gastropoda, Heterobranchia) differ between species that swim using dorsal ventral (DV) or left-right (LR) whole body flexions, even when comparing analogous forms of swimming. To understand molecular elements underlying these differences, we performed deep mRNA sequencing of the central ganglia of six species with each form of swimming behavior, five nudibranchs: *Tritonia diomedea* (DV); *Melibe leonina* (LR); *Hermissenda crassicornis* (LR); *Flabellina iodinea* (LR); and *Dendronotus iris* (LR), and one outgroup *Pleurobranchaea californica* (DV). This effort generated full-length sequences of approximately 24,000 genes, which is similar to the size of the fully sequenced genome of another gastropod, *Lottia gigantea*. Analysis of these sequences is providing broad insights into the molecular elements underlying neuronal excitability, synaptic connectivity, and neuromodulation in these species. For example, we found a disproportionately large number of the most abundantly expressed genes to be secretory neuropeptides (e.g. Cerebral peptide 1, Pedal peptide 1 and Molluscan insulin-related peptide 3), suggesting that peptidergic signaling is a prominent form of neurotransmission in nudibranchs and perhaps all gastropods. Additional insights arise from phylogenetic comparisons: 1) The nudibranch FMRFamide gene possesses considerably fewer repeats of the FMRF peptide motif compared to other molluscs, whereas the *Aplysia* ortholog seems to have duplicated it many more times. 2) The *Melibe* Small Cardioactive Peptide (SCP) gene duplicated to produce a third putative SCP peptide (SCP_C) in addition to SCP_A and SCP_B. We are using the full transcriptome data to resolve ancestral phylogeny of the Nudipleura, to test the hypothesis that DV and LR swimming arose independently in distinct phylogenetic lineages of the Nudipleura. The availability of neuronal gene sequences is expanding research on traditionally electrophysiological preparations to allow a molecular understanding of the basis for species-typical behavior.

Disclosures: **A. Senatore:** None. **P.S. Katz:** None.

Poster

400. Invertebrate Transmission

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 400.09/C36

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF-IOS-1120950

Sigma Xi Grant In Aid of Research

Brains And Behavior Seed Grant

Title: Identification of novel serotonin receptors in six species of sea slugs

Authors: ***A. N. TAMVACAKIS**, A. SENATORE, P. S. KATZ
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Abstract: Serotonin (5-HT) is important for many behaviors in both invertebrates and vertebrates. In sea slugs, 5-HT has been studied extensively with respect to neural control of learning, memory, and movement. To date, four molluscan 5-HT receptor families have been identified and shown to be expressed in neural tissue: 5-HT1, 5-HT2, 5-HT4, and 5-HT7 (Nagakura et al, 2010, J. Neurochem 115, 994-1006). In addition, a putative 5-HT6 receptor family orthologue was identified from *Aplysia* californica genomic DNA. No 5-HT receptors, however, had been identified in any species of Nudipleura sea slugs (Mollusca, Gastropoda, Heterobranchia), which are important models for studying serotonergic neuromodulation. We therefore sequenced the transcriptomes of six sea slug species: Tritonia diomedea, Pleurobranchaea californica, Hermissenda crassicornis, Dendronotus iris, Melibe leonina and Flabellina iodinea. 5-HT receptor homologues from each of the four previously identified molluscan 5-HT receptor families were identified in the transcriptomes of all species. In addition, we confirmed the existence of the 5-HT6 receptor subtype transcript. We also identified a previously uncharacterized 5-HT2 family receptor, 5-HT2b. ClustalX and BLAST alignments showed that 5-HT2b aligned closely with non-molluscan 5-HT2 family receptors, but was distinct from the previously published molluscan 5-HT2 receptor. All transcriptome sequences were consensus sequenced to verify sequence identity. Phylogenetic analyses of the receptor protein sequences showed that they aligned within the distinct molluscan 5-HT receptor families.

Within the receptor sequences, several insertions or deletions resulting in alternative gene sequences were identified in each species. 5-HT-mediated behaviors are likely controlled by multiple 5-HT receptor families; these receptor sequences can therefore be used to study the identity and expression levels of 5-HT receptors involved in many behaviors across multiple species of sea slugs.

Disclosures: A.N. Tamvacakis: None. **A. Senatore:** None. **P.S. Katz:** None.

Poster

400. Invertebrate Transmission

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Program#/Poster#: 400.10/C37

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant R03DA026518

Title: Evidence of nicotine-induced, d-tubocurarine-insensitive behavior in planarians

Authors: *O. R. PAGAN[1], E. MONTGOMERY[2], S. DEATS[2], D. BAKER[2], D. BACH[2]
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Abstract: Planarians are rapidly developing into very useful research subjects in pharmacology and neuroscience research. These organisms possess many of the neurotransmitter systems used by vertebrates and are one of the most primitive example of cephalization in the animal kingdom, displaying a bona fide brain. Here we report that d-tubocurarine, a cholinergic antagonist, alleviates the nicotine-induced planarian seizure-like movements (pSLM) by 50 % up to equimolar concentrations of nicotine and d-tubocurarine (1 mM) while D-tubocurarine alone does not induce pSLM. The simplest interpretation of our data is that there are nicotine induced behaviors insensitive to d-tubocurarine in our experimental organism. To the best of our knowledge, this is the first report on d-tubocurarine-insensitive, nicotine-induced effects.

Disclosures: O.R. Pagan: None. **E. Montgomery:** None. **S. Deats:** None. **D. Baker:** None. **D. Bach:** None.

Poster

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Support: NSF DBI-0932955

NSF DBI 0115825

DoD 52680- RT-ISP

NIMHD 8G12-MD007600 (RCMI)

Title: Identified aminergic neurons in Biomphalaria glabrata, an intermediate host for human intestinal schistosomiasis

Authors: *L. VAASJO[1,2], N. DELGADO[2], S. ROLÓN-MARTÍNEZ[2], M. W. MILLER[2]
[1]Inst. of Neurobio., San Juan, PR; [2]Dept. of Anat. & Neurobio., Inst. of Neurobiology, Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico

Abstract: Schistosomiasis is a neglected tropical disease (NTD) that impacts over 200 million people throughout Asia, Africa and South America. The planorbid snail *Biomphalaria glabrata* serves as the major intermediate host for *Schistosoma mansoni*, the trematode parasite responsible for the most widespread form of human intestinal schistosomiasis. Our previous studies mapped the localization of serotonergic and catecholaminergic neurons in the nervous system of *B. glabrata* (Delgado et al. 2012; Vallejo et al., 2014). We reported the presence of a group of five serotonergic neurons (the CeSF cluster) on the dorsal surface of each cerebral hemiganglion and proposed that this cluster could correspond to a serotonergic system that has been highly conserved in other gastropod groups (see Katz et al. 2001). As these neurons have been intensively studied and found to participate in responses to noxious stimuli in a range of opisthobranch species (*Hermissenda*: CPT cells, *Tritonia:* DSI cells, *Melibe:* Si, *Aplysia*: CC3, *Pleurobranchaea*: As1-3), we explored the possibility that they could be similarly involved in responses to aversive stimuli, such as miracidia penetration, in the pulmonate snail *Biomphalaria*. An isolated "head-brain" preparation was developed to assess the receptive fields of the CeSF neurons to tactile (probe) and noxious (sharp electrode) stimuli designed to simulate penetration of the cephalic epithelium by *S. mansoni* miracidia. We found that neurons comprising the CeSF clusters are anatomically and physiologically heterogeneous. Neurobiotin fills demonstrated dissimilar projections, such as the designated Left Superior (LS CeSF) cell,

projecting contralaterally to the right parietal nerve, while its contralateral counterpart projected bilaterally to multiple peripheral nerves. Experiments demonstrated cells with variable receptive fields, such as the LS cell that was responsive to all areas tested and its contralateral counterpart that was excited only by lip stimulation. Dye fills of some CeSF neurons demonstrated projections to the region of the contralateral CeSF cluster and were indicative of dye coupling. Further experiments were performed to characterize the structure and synaptic relations between the two CeSF clusters and neurons in other ganglia that could support a role for these cells in the responses of *B. glabrata* to noxious or aversive stimuli.

Disclosures: L. Vaasjo: None. **N. Delgado:** None. **S. Rolón-Martínez:** None. **M.W. Miller:** None.

Poster

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Support: NIH Grant AI103790

NSF Grant 1145721

NIH Grant AI15429

Department of Zoology Graduate Research Award

Title: Identification of Neuropeptide-like Proteins (NLPs) in the motor neurons of Ascaris suum

Authors: *J. J. KNICKELBINE, C. J. KONOP, C. D. WRUCK, A. O. W. STRETTON
Univ. of Wisconsin-Madison, Madison, WI

Abstract: The parasitic nematode *Ascaris* affects both humans (*A. lumbricoides*) and livestock (*A. suum*), causing devastating effects on human health and global economy. Treatment involves administration of anthelmintic drugs, which often target classical neurotransmission in the motor nervous system. Detailed studies of cellular connectivity and classical transmitter expression have been completed on the motor nervous system of *Ascaris suum*; however, an adequate functional circuit has not yet been described for this relatively “simple” system. One missing piece of information is the neuropeptide content of the motor neurons themselves. Neuropeptides

are the largest class of neurotransmitter molecules, playing a variety of roles in the nervous systems of vertebrate and invertebrate animals. Neuropeptides are grouped into three categories based on their structure, with the largest and most diverse group being the neuropeptide-like proteins (NLPs). The objective of this study was to identify the neuropeptides produced by the inhibitory and excitatory motor neurons of female *A. suum*, and to examine their effects on classical neurotransmission and locomotory behavior of the worm. To accomplish this, we used single-cell mass spectrometry to determine the peptide content of 5 types of motor neurons. The results were confirmed with *in situ* hybridization. The effects of these peptides on acetylcholine (ACh)-induced muscle contraction were measured on strips of dorsal muscle, and effects on locomotory behavior were observed by injecting intact worms and monitoring their behavior in a tube with the same diameter as the porcine small intestine. We found that the GABAergic inhibitory motor neurons (DI, VI) express only one major peptide product (SLASGRWGLRPamide), which is a homolog to *Caenorhabditis elegans* peptide NLP-22. Pharmacological experiments show a dramatic decrease in ACh-induced muscle contraction of *Ascaris* dorsal muscle in the presence of As-NLP-22, with an IC_{50} of 10^{-8} M. Behavioral experiments in which intact worms were injected with this peptide exhibited flaccid paralysis in the anterior 1/3 of the worm, where locomotory behavior is most frequent. The cholinergic forward-projecting excitatory motor neurons (DE1, DE3, VE1), responsible for forward movement, express 6 peptides homologous to those on the *C. elegans nlp-21* transcript. Muscle exposed to these peptides showed no significant change in ACh-induced contraction compared to controls. Given the dramatic behavioral effects of As-NLP-22, it is crucial that we consider neuropeptides in developing a functional circuit for *Ascaris*, and also as novel targets for anthelmintic drug development.

Disclosures: J.J. Knickelbine: None. **C.J. Konop:** None. **C.D. Wruck:** None. **A.O.W. Stretton:** None.

Poster

400. Invertebrate Transmission

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Program#/Poster#: 400.13/C40

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH

NSF

Title: Cephalopod transcriptomes unravel details about centralized brain evolution across metazoans

Authors: *G. C. WINTERS[1], A. B. KOHN[1], B. HOCHNER[2], N. STERN[2], E. T. WALTERS[3], R. CROOK[3], Y. BOBKOVA[1], L. L. MOROZ[1]
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Abstract: Cephalopod molluscs (e.g. *Nautilus, Loligo, Octopus*, *Sepia*) are powerful models for comparative biology and neuroscience. Their nervous systems range in complexity from simple cords (*Nautilus*) to one of the most intricate brains of the animal kingdom (*Octopus)*. These highly specialized nervous systems allow for an advanced capacity for learning and a rich behavioral repertoire. Of all cephalopod brain innovations, the most extraordinary structure appears to be the vertical lobe, where we find cell circuits modulating the most advanced learning and memory in all invertebrates. Although collaborative efforts have made progress in describing physiological properties of memory circuits, little is known about the implementation of signaling molecules in the vertical lobe. To examine this, we have sequenced neuronal transcriptomes from key model cephalopods and made comparisons to one another and to our sequenced genome and transcriptomes of the gastropod mollusc, *Aplysia californica*. This approach has allowed us to identify evolutionarily conserved neural signaling molecules and numerous innovations within molluscs. For example, we have identified in cephalopods approximately half of all known *Aplysia* neuropeptides, including putative markers for both sensory and motor neuron populations, as well as traditional neuromodulatory peptides. Of all identified cephalopod neuropeptides, we have cloned and localized expression of seventeen in *Octopus vulgaris*, and thirteen in the squid *Loligo pealei*. Of these neuropeptides, about twenty percent appear to localize to specific and distinct cell populations in the vertical lobe, indicating a greater level of complexity than has been previously described. Additionally, some neuropeptides involved in memory modulation in gastropods, such as FMRF-amide, do not localize to the vertical lobe in *Octopus* or *Loligo,* indicating either an alternate role for FMRFamide, or the presence of additional cephalopod memory circuits. Neuropeptide expression in cephalopod and gastropod neural tissues support a hypothesis that there has been expansion of potentially homologous neural cell populations across lineages. This comparative anatomical and genomic approach provides unique opportunities to reconstruct ancestral neuronal lineages, identify conserved cell types across species, and reveal trends in evolution within neural circuits.

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Poster

400. Invertebrate Transmission

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NSF Grant: DBI0115825

Title: Localization of FMRFamide-like immunoreativity in the nervous system of Biomphalaria glabrata, an intermediate host for schistosomiasis

Authors: *R. A. PAGAN-ALEMAN[1,2], S. ROLÓN-MARTÍNEZ[1,2], N. DELGADO-RIVERA[1,2], M. W. MILLER[1,2]
[1]Inst. of Neurobio., Old San Juan, Puerto Rico; [2]Anat. & Neurobio., Univ. of Puerto Rico, Med. Sci. Campus, San Juan, Puerto Rico

Abstract: It is estimated that approximately 10% of people worldwide live at risk of the parasitic disease schistosomiasis, or “snail fever”. The digenetic trematode worm *Schistosoma mansoni* that causes the form of schistosomiasis found in the Western Hemisphere employs the freshwater snail *Biomphalaria glabrata* as its primary intermediate host. As infection of snail hosts by larval trematodes has been reported to alter the expression of genes that encode precursors of molluscan neuropeptides belonging the FMRFamide family (Hoek et al. 1997), this investigation examined the localization of FMRFamide-like immunoreactivity (FMRFa-li) in the central nervous system (CNS) and peripheral nervous system (PNS) of *B. glabrata*. Within the CNS, FMRFa-li neurons were mainly located in the cerebral ganglion (Cer. g.; 34 ± 7), pedal ganglia (Pd. g.; 36 ± 10), left parietal ganglion (L Pa. g.; 18 ± 11) and visceral ganglion (V g.; 13 ± 3). While no FMRFa-li neurons were observed in the buccal ganglion, the buccal neuropil contained branching fibers that originated from axons in the cerebral-buccal connective. In the periphery, structures associated with the male reproductive system (penis muscle and sheath) were richly innervated by FMRFa-li fibers. Double-labeling experiments (biocytin backfill x FMRFa-li) of the penis nerve demonstrated that the neurons projecting to male reproductive structures were

located in the ventral lobe (VL) of the Cer. g. It is suggested that parasite-induced changes in this peptidergic system could contribute to modifications of feeding and reproductive behaviors that have been reported in infected snails.

Disclosures: R.A. Pagan-Aleman: None. **S. Rolón-Martínez:** None. **N. Delgado-Rivera:** None. **M.W. Miller:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.01/C42

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA027990

Title: Differential efficacies of the nicotinic alpha4beta2 desensitizing agents sazetidine-a and analogs in reducing nicotine self-administration in rats

Authors: *E. D. LEVIN[1], S. SLADE[1], C. WELLS[1], A. H. REZVANI[1], Y. LIU[2], V. M. YENUGONDA[2], M. BROWN[2], Y. XIAO[2], K. KELLAR[2]
[1]Duke Univ. Med. Ctr., DURHAM, NC; [2]Georgetown Univ. Sch. of Med., Washington, DC

Abstract: Induced desensitization of neuronal nicotinic acetylcholine receptors holds promise as an effective treatment of tobacco addiction. Previously, we found that Sazetidine-A (Saz-A), which selectively desensitizes alpha4beta2 nicotinic receptors, significantly decreased IV nicotine self-administration (SA) in a rat with an effective dose of 3 mg/kg in acute and chronic studies. We then found that VMY-2-95, a Saz-A analog that we developed, also significantly decreased nicotine SA with the same effective dose. In continuing efforts, we have studied other Saz-A analogs to determine their efficacies in reducing nicotine SA in the rat model.. Young adult female Sprague-Dawley rats were fitted with IV catheters and were trained for nicotine SA (0.03 mg/kg/infusion) on a fixed ratio-1 schedule for ten sessions. Each drug treatment was administered SC in a separate set of rats ten minutes before the start of 45-minute sessions in a counterbalanced design including each dose and saline control twice. YL-2-203, like sazetidine-A and VMY-2-95, significantly reduced nicotine SA at 3 mg/kg. Follow-up investigation with YL-2-203 showed that with chronic administration of 3 mg/kg it was effective in reducing nicotine during two weeks of treatment and during a resumption period after a week-long period of enforced abstinence. VMY-2-109 at 3 mg/kg significantly reduced nicotine SA, but this effect

was restricted to rats with high pre-treatment baseline levels of nicotine SA with no effect discerned in rats with low baseline levels of nicotine SA. YL-1-127 did not have a monotonic dose-effect of reducing nicotine SA; it had some efficacy at a lower (0.3 mg/kg) but not higher (1-9 mg/kg) doses. YL-1-231 did not affect nicotine SA at doses up to 3 mg/kg. Triazetidine-O was not effective in reducing nicotine SA up to 18 mg/kg. These studies, together with our studies of Saz-A and VMY-2-95, revealed a spectrum of efficacies for these alpha4beta2 nicotinic receptor desensitizing agents and provide a path forward for the most effective compounds to be further developed as possible aids to smoking cessation.

Disclosures: **E.D. Levin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **S. Slade:** None. **C. Wells:** None. **A.H. Rezvani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **Y. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **V.M. Yenugonda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **M. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **Y. Xiao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents. **K. Kellar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.02/C43

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA U19 grant DA027990

NIAAA R24-AA015512-02

Title: Role of nicotinic acetylcholine α4β2* receptors in alcohol intake: testing novel compounds in rats

Authors: *A. H. REZVANI[1], M. L. BROWN[2], Y. XIAO[2], Y. LIU[2], V. M. YENUGONDA[2], K. J. KELLAR[2], E. D. LEVIN[1]
[1]Dept. of Psychiatry, Duke Univ., Durham, NC; [2]Georgetown Univ., Washington DC, DC

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) have been implicated in alcohol drinking behavior and manipulation of these receptors has been shown to influence alcohol consumption. The goal of the present study was to examine the effects of a series of novel compounds that desensitize α4β2* nAChRs on alcohol intake in alcohol preferring (P) rats. Sazetidine-A (Saz-A), VMY-2-95, YL-1-127 and YL-2-203 were tested. These compounds are strong desensitizers of α4β2 receptors and demonstrate only weak varying partial agonist effects. Alcohol preferring rats were given the choice of water or alcohol. Once stable baselines were established, the acute effects of Saz-A (0.1, 0.3, 1 and 3 mg/kg, s.c.) and its chronic effect (3 mg/kg for 10 days) as well as the effects of different doses of VMY-2-95 (0.33, 1 and 3 mg/kg), YL-1-127 (0.33, 1, 3, 6 and 9 mg/kg) and YL-2-203 (0.33, 1, 3 and 6 mg/kg) on alcohol intake and preference were assessed. In addition, the acute effects of Saz-A (3 mg/kg) and YL-1-203 (6 mg/kg) and VMY-2-95 (3 mg/kg) on alcohol intake after alcohol deprivation were evaluated. The saline vehicle was used as control. Alcohol intake and preference were recorded at 2, 4, 6 and 24 h after the treatments. Our results show that the lead compound Saz-A caused a dose-dependent reduction in alcohol intake and preference with an effective dose threshold at 1 mg/kg). Chronic Saz-A also effectively reduced alcohol intake. In the post-deprivation experiment, when the urge for drinking is enhanced, Saz-A significantly reduced alcohol intake and preference. YL-1-127 at 1 mg/kg reduced alcohol intake significantly over 24-h, and at 3 mg/kg the compound reduced alcohol intake at both the 6-h and 24-h. VMY-2-95 at all doses tested reduced alcohol intake at all-time points. VMY-2-95 at 3 mg/kg also significantly reduced alcohol intake after alcohol deprivation. YL-2-203 was not effective in reducing alcohol intake. In conclusion, these findings suggest an important role for α4β2 nAChRs in excessive drinking and indicate that compounds that desensitize these receptors may hold promise for the treatment of alcohol use disorders. (Supported by NIDA U19 grant DA027990 and NIAAA R24-AA015512-02).

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Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.03/C44

Topic: B.02. Ligand-Gated Ion Channels

Support: NAAA grant AA020082

Title: Cross tolerance between nicotine and ethanol is accompanied by increased ethanol self-administration and changes in striatal synaptic transmission

Authors: C. ABBURI[1], R. A. E. METZ[1], R. KAMBER[1], S. L. WOLFMAN[2], D. S. MCGEHEE[2,1], *J. MCDAID[1]
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Abstract: Nicotine and ethanol (EtOH) are two of the most widely abused and co-abused drugs. A major risk factor for alcoholism is tolerance to intoxication as this may result in greater EtOH consumption and greater likelihood of addiction. In a number of animal studies, increased EtOH self-administration is correlated with tolerance to EtOH intoxication. Although nicotine EtOH cross-tolerance has been demonstrated in a number of studies, this has not been correlated with changes in levels of EtOH self-administration. In this study we demonstrate that intermittent nicotine pretreatment in Sprague-Dawley rats results in tolerance to nicotine induced motor impairment and cross tolerance to EtOH induced motor impairment, as assessed using the accelerating rotarod. This cross tolerance to EtOH is accompanied by persistently increased levels of EtOH self-administration using a 20% EtOH solution in a 2-bottle choice test. The dorsolateral striatum (DLS) has been implicated in both reward and motor control. Using electrophysiology, we assessed the effects of repeated nicotine on excitatory synaptic transmission in DLS medium spiny neurons (MSN's). Using the paired-pulse ratio as a measure of the release probability of glutamate, we found that repeated nicotine increased the paired-pulse ratio in DLS MSN's, indicating a decrease in the release probability of glutamate. Further studies will assess the role of nicotine induced changes in DLS excitatory synaptic transmission in both EtOH tolerance and reward.

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Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

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Program#/Poster#: 401.04/C45

Topic: B.02. Ligand-Gated Ion Channels

Title: Interactions of hippocampal nAChR subtypes with beta amyloid and the kinase inhibitor gesistein

Authors: D. G. CLARK[1], *S. N. SUDWEEKS[2]
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Abstract: The exact mechanism and progression of Alzheimer's disease (AD) at present is not fully understood. Damage to the hippocampal region in AD is evidenced by the impairment of learning and memory. It is also known that a buildup of β-amyloid plaques occur in AD patients and that β-amyloid interacts with some subtypes of neuronal nicotinic acetylcholine receptors (neuronal nAChRs). The neuronal nAchRs are pentameric and can consist of several different subunit types. The most prevalent nAChR in the brain as a whole are homomeric α7 receptors and heteromeric receptors composed of α4 and β2 subunits. A broad variety of neuronal nAChR subunits are known to be expressed by interneurons within the hippocampus, a brain region strongly associated with learning and memory. It is hypothesized that memory formation can be impaired through the interaction of β-amyloid with these nAChRs. In this experiment we characterize the interactions of β-amyloid$_{42}$ with human nAChR subunit combinations expressed in *Xenopus laevis* oocytes. We observed a decrease in potentiation when the homomeric α7 receptors was exposed to β-amyloid42. We also observed genistein, a kinase inhibitor, modulates the effects of β-amyloid42 on the α7 receptors, indicating a possible therapeutic option to combat the neurodegeneration in AD.

Disclosures: D.G. Clark: None. **S.N. Sudweeks:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

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Travis Denton's research was supported by the Eastern Washington University Foundation

Title: Novel myosmine derivatives as potential therapeutics for Alzheimer's disease evaluated *in vitro* by multi-electrode electrophysiology of a neuronal network and X-ray crystallography

Authors: *T. T. DENTON[1,2], T. T. TALLEY[3], P. SENGUPTA[4], K.-Y. HO[5], J. BOBANGO[3], B. MUMMEY[2], A. LE[2], A. PENTECOST[2], P. TAYLOR[5]
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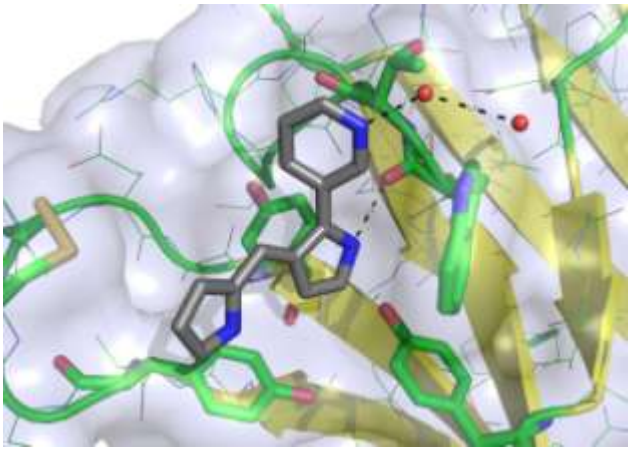
Abstract: Nicotinic acetylcholine receptors (nAChRs) are recognized for playing a pivotal role in cognitive function. Signaling between cholinergic neurons has been shown to be compromised in many models of Alzheimer's disease. Stimulation of nAChRs with synthetic analogues of the natural neurotransmitter has been shown to reinvigorate cognitive function in such models. In order to identify new chemical entities to potentially aid in the treatment of Alzheimer's disease, a panel of myosmine analogues was prepared and screened with a high throughput method utilizing a series of acetylcholine binding proteins (AChBPs). Some of the compounds prepared had affinities for the AChBPs in the low nanomolar range. Select compounds were co-crystallized with the AChBPs and X-ray structures obtained help demonstrate essential molecular interactions within the binding pocket. The highest affinity compound from the set was studied for its toxicity and efficacy using multi-electrode electrophysiology of dissociated neuronal networks made with hippocampal neurons from c57bl6/J mice. The results of these assays show that the compound was not toxic to the neuronal network and it modified synaptic activity in a dose dependent manner. This data provides the necessary information to extend the testing of the potent compounds in networks mimicking Alzheimer's brain conditions.



Disclosures: **T.T. Denton:** None. **T.T. Talley:** None. **P. Sengupta:** None. **K. Ho:** None. **J. Bobango:** None. **B. Mummey:** None. **A. Le:** None. **A. Pentecost:** None. **P. Taylor:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.06/C47

Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC Discovery Grant (CDCB)

Title: Developmental regulation of nicotinic receptor signaling in mouse hippocampal CA1 pyramidal neurons

Authors: B. Y. T. CHUNG[1], D. L. JACKLIN[2], W. BIGNELL[1], B. D. WINTERS[2], *C. D. BAILEY[1]
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Abstract: The normal development and function of the hippocampus depends on afferent cholinergic neurotransmission mediated by nicotinic acetylcholine receptors (nAChRs). Nicotinic signaling during development has been implicated in shaping the long-term dendritic morphology of neurons in the central nervous system, and specifically within the hippocampus has been demonstrated to persistently influence the number of postsynaptic spine synapses on CA1 pyramidal output neurons. A major class of nAChR in the hippocampus is the α4β2* receptor. Although it is established that α4β2* nAChRs mediate nicotinic signaling in hippocampal GABAergic interneurons, their ability to mediate nicotinic signaling in glutamatergic CA1 pyramidal neurons is not well understood. Interestingly, expression of individual α4β2* receptor subunits within the rodent CA1 pyramidal cell layer is developmentally regulated and greatest during early postnatal life, suggesting that receptor function is greatest in these neurons at this time. We sought to determine whether functional α4β2* nAChRs are present on CA1 pyramidal neurons during the postnatal development of male CD1 strain mice. Whole-cell electrophysiological responses to acetylcholine (ACh) were recorded for visually-identified CA1 pyramidal neurons within acute brain slices, in the presence of both atropine (to block muscarinic acetylcholine receptors) and methyllycaconitine (MLA; to block α7 subunit-containing nAChRs). We found that ACh elicited postsynaptic inward currents and facilitated neuronal excitation, and that the magnitude for both of these nicotinic responses was greatest in CA1 pyramidal neurons during the first and second postnatal weeks of life compared with later developmental ages examined. Nicotinic responses were resistant to antagonists of synaptic transmission and were inhibited by dihydro-β-ethroidine, suggesting that they were mediated by α4β2* nAChRs located directly on recorded CA1 pyramidal neurons. These findings demonstrate that α4β2* nAChRs mediate nicotinic signaling in CA1 pyramidal

neurons during hippocampal development, and suggest a role for these receptors in the formation and maturation of hippocampal learning and memory networks.

Disclosures: B.Y.T. Chung: None. **D.L. Jacklin:** None. **W. Bignell:** None. **C.D. Bailey:** None. **B.D. Winters:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.07/C48

Topic: B.02. Ligand-Gated Ion Channels

Title: Facilitation of alpha 7 nachr current by picomolar concentration of amyloid beta peptide

Authors: *M. ISLAM, K. DEBOEUF, L. LAURIDSEN, J. BAE, J. FARLEY
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Abstract: Alpha 7 (α7) nicotinic acetylcholine receptors (nAChRs) are widely distributed in brain particularly in those areas involved in motivation, learning and memory. Various interactions of α7 Rs with amyloid beta peptides (e.g., Aβ 42) have been proposed by several groups, with possible relevance for Alzheimer's disease (AD). The results have been variable and mixed, however, and no clear consensus has yet been reached concerning their interaction or functional significance. Early studies (Wang et al, 2000, J. Neurochem) showed that α7 nAChRs and Aβ 42 are co-localized in AD cortical regions including the hippocampus, and further that high-affinity receptor-peptide complexes could be co-immunoprecipitated (Wang et al., 2010, Biol Psychiatry). The high affinity interaction of Aβ 42 with α7 Rs may be unique to low-n oligomeric forms of Aβ, but the exact nature of the interaction is still largely unknown (Parri et al, 2011, Biochem. Pharmacol.). The apparent high affinity for the α7 R-Aβ 42 interaction suggests that the very low (pM) concentrations of Aβ 42 found in healthy brain under normal physiologic conditions (Kuo et al, 1996, J Biol Chem) might facilitate receptor activation by ACh. In support of this view, behavioral and synaptic plasticity studies in hippocampus found that pM Aβ 42 was essential for normal function of alpha 7 Rs (Puzzo et al, 2010, Ann Neurol). In contrast, high (nM - uM) concentration of Aβ 42 found in advanced AD patient brain (Kuo et al, 1996, J Biol Chem) might have an inhibitory effect on α7 nAChRs as suggested by some studies. To elucidate the functional consequences of Aβ 42 - α7 nAChR interactions, we tested the effects of Aβ 42 (1 pM, 10 pM, 1 uM applied acutely as well as for several hrs) on murine α7 nAChRs heterologously expressed in Xenopus oocytes. No concentration of Aβ 42 tested was

found to directly activate α7 Rs. Similarly, short term preexposure (10 sec) to Aβ 42 (10 sec) produced no effect on 500 uM ACh-evoked α7 currents (i.e., neither PAM- nor NAM-like effects observed). However, incubation with 10 pM Aβ for 2-4 hours produced a clear and significant ~ 3-fold facilitation of α7 currents (mean ± SEM) : Controls, 242±24 nA (n=36) vs. 10 pM, 742±190 nA (n=35). Lower or higher Aβ 42 concentrations had no significant effects: 1 pM, 219±39 nA (n=18); 1 uM, 245±29 nA (n=19). Thus, low Aβ 42 concentrations (similar to physiologic conc. in brain) facilitated α7 function. Additional studies examined the relative contribution of Type I PAM-like actions vs. intracellular signaling pathways to the facilitatory effects of 10 pM Aβ 42 on α7 currents.

Disclosures: M. Islam: None. **K. DeBoeuf:** None. **L. Lauridsen:** None. **J. Bae:** None. **J. Farley:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.08/C49

Topic: C.17. Drugs of Abuse and Addiction

Support: Academy of Finland

Title: Association of alcohol preference with the low hypothalamic alpha7 nicotinic receptor level

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Abstract: Nicotine and alcohol are often used together. The effects of nicotine are mediated via neuronal nicotinic receptors in the brain. In addition, emerging evidence suggests that nicotinic receptors are involved in the mediation of the rewarding and reinforcing effects of alcohol. Here we studied the expression of α5, α6 and α7 nicotinic receptor subunits in alcohol preferring AA and alcohol non-preferring AA rats. *In situ* hybridization and receptor binding studies were conducted in drug-naïve animals. The brain areas to be studied were selected based on their high expression level of these nicotinic receptor subunits and if earlier association of behaviors linked to alcohol or nicotine dependence with these areas was suggested. We found that the mRNA level of α5 subunits in the medial habenula and hippocampus were similar in AA and ANA rats.

In addition, there was no difference in the expression of α6 subunit mRNA in the ventral tegmental area and substantia nigra. However, the level of α7 nicotinic receptor subunit mRNA was significantly lower in the hypothalamus of alcohol preferring AA rats than in alcohol avoiding ANA rats. Also the hypothalamic [125]I-α-bungarotoxin binding was lower in AA rats indicating lower levels of α7 nicotinic receptors. Hippocampal α7 subunit mRNA level and receptor binding for α7 nicotinic receptors were similar in AA and ANA rats. The α7 nicotinic receptors in the hypothalamus are located in histaminergic neurons suggesting that regulation of genetic preference or avoidance to alcohol or other addictive substances involves brain histaminergic system.

Disclosures: S. Nuutinen: None. **O.S. Salminen:** None. **P. Panula:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA030396

NIH Grant GM103801

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Ralph W. and Grace M. Showalter Research Trust

Frederick N. Andrews Fellowship

Title: Combinations of nicotine and ethanol enhance AMPA receptor function in VTA DA neurons through activation of α6β2* nAChRs

Authors: *S. E. ENGLE[1], J. M. MCINTOSH[2,3], R. M. DRENAN[1]
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Abstract: Nicotine and alcohol are frequently co-abused, suggesting they may have common molecular targets mediating their rewarding effects. While nicotine can directly activate nicotinic

acetylcholine receptors (nAChRs) in the mesolimbic dopamine (DA) system, emerging evidence suggests that alcohol can modulate nAChR function. nAChRs containing the α6 subunit are highly and selectively expressed in midbrain DA neurons of the substantia nigra and ventral tegmental area (VTA). We previously reported that nicotine can act selectively through α6β2* nAChRs to enhance 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA) receptor (AMPAR) function on VTA DA neurons. This effect sensitizes the VTA to excitatory input and promotes drug seeking in animal models. Exposure to alcohol also enhances AMPAR function in VTA neurons. We therefore hypothesized that α6β2* nAChRs are also involved in the mechanism by which ethanol enhances AMPAR function on VTA DA neurons. Mice expressing hypersensitive α6 nAChRs (α6L9′S mice) were used in this study to determine if enhanced α6* nAChR activity can modulate the ability of ethanol to enhance AMPAR function in VTA DA neurons. We found that incubating naïve brain slices containing the VTA in a low concentration of ethanol (5 mM) for 60 min was sufficient to enhance AMPAR function in VTA DA neurons of α6L9′S mice. This concentration of ethanol was insufficient to enhance AMPAR function in non-transgenic littermate neurons, but 20 mM ethanol was sufficient to enhance AMPAR function in these control mice. To determine whether *in vivo* ethanol exposure produced effects similar to those seen in experiments utilizing slice incubation, α6L9′S and nonTg littermate mice were injected (i.p.) with ethanol and 60 min later brain slices were prepared. α6L9′S mice injected with 0.5 g/kg ethanol exhibited enhanced VTA DA neuron AMPAR function, but nonTg control mice required 2.0 g/kg ethanol to achieve the same effect. Because ethanol and nicotine both modulate AMPAR function in a manner involving α6* nAChRs, we tested the hypothesis that low concentrations of alcohol and nicotine combine to modulate AMPAR function in an additive manner. Co-incubation of α6L9′S or nonTg slices in concentrations of ethanol and nicotine that are sub-threshold when incubated alone resulted in robust enhancement of AMPAR function. Pre-incubation of slices in either α-conotoxin MII (α6β2* nAChR antagonist) or varenicline (a partial agonist of α4β2* and α6β2* nAChRs) attenuated the additive/synergistic enhancement of low concentrations of nicotine and alcohol. These data suggest that α6β2* nAChRs play an important role in alcohol+tobacco co-dependence.

Disclosures: **S.E. Engle:** None. **J.M. McIntosh:** None. **R.M. Drenan:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.10/C51

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031791

Title: Individual differences in the modulation of rapid dopamine signals by presynaptic nicotinic acetylcholine receptors

Authors: *M. J. FERRIS, S. R. JONES
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Abstract: Variability in the rate in which animals will acquire self-administration of psychostimulants is a preclinical model for vulnerability to abuse drugs in humans. The variability in animals' acquisition rate can be predicted by individual differences in animals' locomotor response to a novel environment. Therefore, we investigated whether the propensity to explore a novel environment might also predict individual differences in the activity of receptors that have been shown to be critical for acquisition behavior. We focused on nicotinic acetylcholine receptors (nAChR) located on dopamine terminals in the core of the nucleus accumbens given their well-documented ability to modulate rapid dopamine signals that are critical for acquisition behavior. Moreover, activation of nAChRs is critical for amphetamine, cocaine, and nicotine sensitization, and nAChR blockade reduces psychostimulant self-administration. We assessed locomotor activity in an inescapable novel environment followed by measurements of dopamine release using voltammetry in brain slices from the same animals. We found no relationship between the animals' locomotor response to a novel environment and the magnitude of electrically-stimulated dopamine release in the nucleus accumbens. In fact, no relationship between these parameters was found when dopamine was elicited under single-pulse conditions and under multiple-pulse conditions across a range of frequencies (5 Hz - 100 Hz). Following assessment of dopamine release under drug-free conditions, the general nAChR antagonist mecamylamine, the β2-specific antagonist DHβE, and a desensitizing dose of nicotine was bath applied to different slices from the same animal. Animals with lower response to a novel environment (LR) showed greater inhibition in dopamine release to nAChR blockade or desensitization under single pulse and low frequency, multiple-pulse stimulations that reflect tonic firing of dopamine neurons. However, animals with a higher response to a novel environment (HR) showed greater facilitation of dopamine release to nAChR blockade or desensitization under multiple-pulse, high-frequency conditions that reflect phasic firing of dopamine neurons. Thus, LR animals are more sensitive to the dopamine inhibitory effects of nAChR blockade under tonic firing-like conditions while HR animals are more sensitive to the dopamine facilitative effect of nAChR blockade under phasic firing-like conditions. The increased sensitivity to nAChR induced facilitation of dopamine signals elicited by phasic-like stimulation parameters may be mechanistically linked to faster and more robust acquisition of drugs of abuse.

Disclosures: **M.J. Ferris:** None. **S.R. Jones:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.11/C52

Topic: B.02. Ligand-Gated Ion Channels

Support: This work was supported by Eli Lilly and Company through the Lilly Research Award Program (LRAP)

Title: Native α7-containing nicotinic acetylcholine receptors form heteromers with β2 subunits in the human cortex and have a distinct pharmacology: Evidence for an alternative drug target for schizophrenia and Alzheimer's Disease

Authors: *J. D. MIKKELSEN[1], R. ZWART[2], D. URSU[2], G. GILMOUR[2], M. M. JENSEN[1], L. PINBORG[1], J. WU[3], E. SHER[2], M. S. THOMSEN[1]
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Abstract: In the brain, the α7 nicotinic acetylcholine receptor (nAChR) is composed as a penta-homomeric ligand gated ion channel. In addition, α7β2 nAChR heteromers have been demonstrated in heterologous expression systems. Since partial agonists currently used in clinical trials are selected from screening on the homomeric and not the heteromeric receptor, it is important to determine whether α7β2 nAChRs are present in the human brain, and whether such receptors are pharmacologically different from α7 nAChR homomers. In this study, we used α-bungarotoxin to affinity purify α7-containing nAChRs from surgically excised human temporal cortex, and determined the pharmacology and kinetics of human α7β2 nAChR heteromers in two cellular systems. Using α-bungarotoxin pull down from extracts of temporal neocortex resected under neurosurgical operations, it was revealed that β2 subunits co-purified in the eluent. The same data were replicated in three distinct human samples. In animal studies, the co-purification of β2 occurs in the cortical tissue from wild-type, but not α7 or β2 knock-out mice. These data strongly indicate that the α7β2 nAChR heteromer is indeed present in the human brain. Furthermore, the pharmacology and kinetics of human α7β2 nAChR heteromers differ significantly from that of α7 homomers in response to the nAChR ligands carbachol, choline, epibatidine and compound A when expressed in Xenopus oocytes and HEK293 cells. Notably, α7β2 heteromers expressed in HEK293 cells display markedly slower rise and decay phases in response to epibatidine and compound A. The present results demonstrate that part of the α7-containing nAChRs in the human brain are heteromers containing one or more β2 subunits, and

that human α7β2 nAChR heteromers respond to selective α7 nAChR agonists with unique pharmacology and kinetic profiles. The α7β2 nAChR heteromer thus represents an alternative mechanism through which the reported clinical efficacy of α7 nAChR ligands may occur, and may represent an alternative drug target for treatment of cognitive dysfunction.

Disclosures: **J.D. Mikkelsen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eli Lilly & Co. **R. Zwart:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **D. Ursu:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **G. Gilmour:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **M.M. Jensen:** None. **L. Pinborg:** None. **E. Sher:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Wu:** None. **M.S. Thomsen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eli Lilly & Co.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.12/C53

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA012976

Title: The associations of the α5 neuronal nicotinic acetylcholine receptor subunit in the rodent habenula

Authors: ***R. VENKATESH**, R. P. YASUDA, T. H. GUPTA-GOLDENBERG, B. B. WOLFE, K. J. KELLAR
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) play a pivotal role in nicotine addiction. Heteromeric neuronal nAChRs are ligand-gated, pentameric, cation channels formed from combinations of 9 α and 3 β subunits, which allows for the possibility of a large number of receptor subtypes with different biophysical properties and pharmacological characteristics. The habenula is an important brain region that expresses several different nAChR subtypes (Grady et al., J Neurosci, 2009; Fonck et al., Neuropharmacology, 2009), some of which appear to be closely linked to withdrawal symptoms in nicotine-treated animals (Salas et al., J Neurosci, 2009) Many of the nAChRs in the habenula contain the α5 subunit, which appears to play an important role in nicotine addiction. The α5-containing receptors have significantly higher Ca++

conductance and faster rates of desensitization than their non α5-containing counterparts (Gerzanich et al., JPET, 1998). Additionally, the α5 subunit in the habenula may play a role in animals' sensitivity to high doses nicotine and their threshold for aversion (Fowler et al., Nature 2011). Although many nAChR subtypes are expressed in the rat habenula, the distribution of the a5 subunit in the different subtypes, e.g., α3β4 and α4β2 nAChRs, is still not certain. In this study, we investigated the associations of the α5 subunit in the rat habenula using the sequential immunoprecipitation method. This method allowed us to quantify the total α5 subunit expression in the habenula, as well as the extent to which α5 was associated with other subunits. Our study shows that the α5 subunit exists in about 20% of the nAChRs that are expressed in the rat habenula. Additionally, we show that in the rat habenula the α5 subunit is associated with both β2- and β4-containing receptors, providing the habenula with a diverse group of α5-containing nAChRs. This data provides important insight into how α5 can potentially mediate functions of the habenula by incorporating into multiple nAChR subtypes. By understanding the library nAChR subtypes in the habenula, it may be possible to understand how the actions of these nAChRs impact nicotine addiction. Therefore, this study will provide important insight into the role habenular nAChRs play in addiction.

Disclosures: R. Venkatesh: None. **R.P. Yasuda:** None. **T.H. Gupta-Goldenberg:** None. **B.B. Wolfe:** None. **K.J. Kellar:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.13/C54

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R21DA031952

NIH Grant R21DA033543

NIH Grant F31DA029386

NIH Grant F30DA036312

Title: miRNome analysis of the mammalian neuronal nicotinic acetylcholine receptor gene family

Authors: A. P. CASSERLY[1], E. M. HOGAN[1], M. D. SCOFIELD[1], Z. MOU[1], R. ZHAO-SHEA[1], C. W. JOHNSON[1], A. R. TAPPER[1], *P. D. GARDNER[2]
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Abstract: Nicotine binds to and activates a family of ligand-gated ion channels, neuronal nicotinic acetylcholine receptors (nAChRs). Chronic nicotine exposure alters the expression of various nAChR subtypes, which likely contributes to nicotine dependence; however, the underlying mechanisms regulating these changes remain unclear. A growing body of evidence indicates that microRNAs (miRNAs) may be involved in nAChR regulation. Using bioinformatics, miRNA library screening, site-directed mutagenesis and gene expression analysis, we have identified a limited number of miRNAs that functionally interact with the 3′-untranslated regions (3′-UTRs) of mammalian nAChR subunit genes. In silico analysis revealed specific, evolutionarily conserved sites within the 3′-UTRs through which the miRNAs regulate gene expression. Mutating these sites disrupted miRNA regulation confirming the in silico predictions. In addition, the miRNAs that target nAChR 3′-UTRs are expressed in mouse brain and are regulated by chronic nicotine exposure. Furthermore, we show that expression of one of these miRNAs, miR-542-3p, is modulated by nicotine within the mesocorticolimbic reward pathway. Importantly, over-expression of miR-542-3p led to a decrease in the protein levels of its target, the nAChR β2 subunit. Bioinformatic analysis suggests that a number of the miRNAs play a general role in regulating cholinergic signaling. Our results provide evidence for a novel mode of regulation of the mammalian nAChR gene family by nicotine.

Disclosures: A.P. Casserly: None. **E.M. Hogan:** None. **M.D. Scofield:** None. **Z. Mou:** None. **R. Zhao-Shea:** None. **P.D. Gardner:** None. **A.R. Tapper:** None. **C.W. Johnson:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

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Program#/Poster#: 401.14/C55

Topic: B.02. Ligand-Gated Ion Channels

Support: Danish Strategic Research Council (COGNITO)

Lundbeck Foundation

Lilly Research Award Program

Title: Lynx1 interacts with multiple nicotinic acetylcholine receptor subtypes in the human brain: Relation to Alzheimer's disease-like pathology

Authors: *M. S. THOMSEN[1], M. ARVANITI[2], M. M. JENSEN[2], E. N. LYUKMANOVA[3], M. A. SHULEPKO[3], W. HÄRTIG[4], V. TSETLIN[3], J. D. MIKKELSEN[2]
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Abstract: Ly6/neurotoxin 1 (Lynx1) is an important regulator of synaptic plasticity in the brain, and this is believed to be due to its regulation of nicotinic acetylcholine receptors (nAChRs). Heterologous expression studies have shown that Lynx1 binds directly with α7 and β2 nAChR subunits, but it is not known to which extent Lynx1 can bind to endogenous nAChRs in the brain or how this is affected by Alzheimer's disease pathology. Here, we apply affinity purification with recombinant soluble Lynx1 (Ws-Lynx1) to show that Ws-Lynx1 can co-purify α4, α5, α6, α7, β2, and β4 nAChR subunits from human cortical extracts as well as rat cortical, midbrain and olfactory bulb extracts. We further show that Ws-Lynx1 can decrease nicotine-mediated Extracellular-signal Regulated Kinase (ERK) phosphorylation in rat PC12 cells. This finding indicates that binding of Lynx1 is sufficient to inhibit signalling downstream of nAChRs. The effect of nicotine itself in this assay is sensitive to inhibition by mecamylamine, but not by methyllycaconitine or dihydro-β-erythroidine. This suggests that Lynx1 can affect the function of native non-α7, non-α4β2 nAChR subtypes. Finally, we show that oligomeric β-amyloid1-42 can inhibit the interaction between Ws-Lynx1 and several nAChR subunits, and that cortical Lynx1 levels are decreased in a transgenic mouse model with age-dependent β-amyloidosis and tau pathology. Our data suggest that Lynx1 is a promiscuous protein that can bind to and regulate multiple nAChR subtypes in the human brain, and implicates Lynx1 in the pathophysiology of Alzheimer's disease.

Disclosures: **M.S. Thomsen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); This study was partly funded by Eli Lilly and Company through the Lilly Research Award Program (LRAP). **M. Arvaniti:** None. **M.M. Jensen:** None. **E.N. Lyukmanova:** None. **M.A. Shulepko:** None. **V. Tsetlin:** None. **J.D. Mikkelsen:** None. **W. Härtig:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

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Program#/Poster#: 401.15/C56

Topic: B.02. Ligand-Gated Ion Channels

Support: NS59910 [MQ]

NS65851 [MQ]

DE030396 [RMD]

Ralph W. and Grace M. Showalter Research Trust [RMD]

Title: Blockade of α6* nAChRs reduces L-dopa-induced dyskinesias; studies using parkinsonian α6* nAChR gain of function mice

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Abstract: L-Dopa-induced dyskinesias (LIDs) are a serious side effect of dopamine replacement therapy for Parkinson's disease for which there are a few treatment options. Accumulating preclinical studies using nicotinic agonists and nicotinic receptor (nAChR) null mutant mice suggest a role for various nAChRs in LIDs, including the α6β2* nAChR population. Deletion of the α6 nAChR subunit reduced L-dopa-induced abnormal involuntary movements (AIMs) compared to wild type (WT) mice, while nicotine treatment reduced AIMs in WT but not in α6 nAChR knockout mice. To understand the mechanism through which α6β2* nAChRs regulate L-dopa-induced AIMs, we used gain-of-function α6* nAChR (α6L9'S) mice. This mutation results in enhanced sensitivity to nicotine/acetylcholine and prolonged receptor-mediated activity. Male WT and α6L9'S mice were lesioned by unilateral injection of 6-hydroxydopamine (3 µg/ml) into the medial forebrain bundle. Three to 4 wk later, they were rendered dyskinetic by administration of L-dopa (3 mg/kg) plus benserazide (15 mg/kg) for 3 wk. L-dopa-induced AIMs were expressed to a similar extent in the α6L9'S mice and WT mice. Three week later, when AIMs had stably developed, α6L9'S mice and WT mice were administered nicotine. WT mice were given nicotine in the drinking water in gradually increasing doses to a maximum of 300 µg/ml. This dose led to a 40% decline in L-dopa-induced AIMs. The reduction in AIMs in WT mice was maintained with doses of nicotine as low as 20 µg/ml. Because of their exquisite sensitivity to nicotine, α6L9'S mice received 10 µg/ml in the drinking water; higher nicotine doses resulted in mortality. In contrast to WT mice, nicotine treatment did not reduce L-dopa-induced AIMs in α6L9'S mice. To determine whether this may be due to enhanced activity of hypersensitive α6L9'S nAChRs, we tested the effect of the general nAChR blocker mecamylamine that has previously been shown to reduce L-dopa-induced AIMs. Two-day administration of mecamylamine (1 mg/kg ip 30 min before L-dopa) similarly reduced L-dopa-induced AIMs in

α6L9'S mice and WT mice. The observation that a nAChR antagonist still reduced L-dopa-induced AIMs in α6L9'S mice suggests that nicotine decreases LIDs by a nAChR desensitization block. These results indicate that α6β2* nAChR antagonists may be useful for reducing L-dopa-induced dyskinesia.

Disclosures: M.M. McGregor: None. **M. Quik:** None. **T. Bordia:** None. **R.M. Drenan:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.16/C57

Topic: C.17. Drugs of Abuse and Addiction

Support: Institut Pasteur

PPU International PhD Programme

Stavros Niarchos Foundation

CNRS

Title: Role of nicotinic receptor subunits in spontaneous activity of prelimbic cortex

Authors: F. KOUKOULI[1], D. TZIOTIS[2], M. NILGES[2], D. DIGREGORIO[1], *U. MASKOS[3]
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Abstract: The Prefrontal Cortex (PFC) is important for many cognitive processes including attention, decision making, social behavior and emotion. The PFC receives significant cholinergic innervation in concert with numerous neurons in this brain region expressing nicotinic Acetylcholine Receptors (nAChRs). nAChRs are pentameric transmembrane proteins that exhibit divergent cationic permeabilities, agonist affinities and desensitization properties. In our study, we investigated *in vivo* the cholinergic modulation of PFC layer II/III neurons in order to better understand the impact of specific nAChR subunits on neuronal network activity. We performed two-photon Ca2+ imaging in lightly anesthetized, adult, wild type and subunit-specific knock-out (KO) mice (α7, β2, α7β2 and α5). In WT mice (6-8 months old) we categorized neuronal firing behavior according to the number of Ca2+ events per minute (EPM) as segregated with principal component analysis. Silent cells produced 1 or less EPM, whereas

moderately firing neurons produced between 1 to 34 EPM, and finally hyperactive neurons produced more than 34 EPM. The distribution of spontaneous activity was remarkably stable in WT mice, with 71 ± 3% having normal EPM and only 7 ± 3% showing hyperactive EPM. In all the various nAChR subunit KO mice, the distribution of firing types was altered as compared to WT. A common finding was a significant increase in the fraction of hyperactive neurons, with a notable three-fold increase in the a7 KO mice. Interestingly, we did not observe significant differences in the fraction of silent neurons between WT mice and β2 and α5 KO mice. In order to assess the effect of acute alterations in nAChR signaling on PFC activity, we subcutaneously administered nicotine and other subunit-specific cholinergic agonists while imaging. Our findings suggest that nicotine regulates PFC baseline neuronal activity and is dependent on the nAChR subunit composition.

Disclosures: F. Koukouli: None. **D. DiGregorio:** None. **U. Maskos:** None. **D. Tziotis:** None. **M. Nilges:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Program#/Poster#: 401.17/C58

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Support: NS59910 (MQ)

NS65851 (MQ)

GM57481 (RLP)

GM103801 (JMM)

GM48677 (JMM)

Title: The α7 nicotinic receptor agonist ABT-107 protects against nigrostriatal damage in parkinsonian rats

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Florida Col. of Med., Gainesville, FL; [3]George E. Wahlen Veterans Affairs Med. Ctr., Salt Lake City, UT; [4]AbbVie, Inc, 1 North Waukegan Road, North Chicago, IL

Abstract: The finding that smoking is inversely correlated with Parkinson's disease and that nicotine attenuates nigrostriatal damage in parkinsonian animals supports the idea that nicotine may be neuroprotective. Nicotine is thought to exert this effect by acting at nicotinic receptors (nAChRs), including the α7 nAChR subtype. The objective of this study was twofold; first, to test the neuroprotective potential of ABT-107, an agonist with high affinity and selectivity for α7 nAChRs and has low incidence of side effects in patients, and second, to investigate its mechanism of action. Rats were implanted with minipumps containing ABT-107 (0.25 mg/kg/d). In addition, we tested the effect of the α7 nAChR agonist DMXB (2 mg/kg/d) and nicotine (1 mg/kg/d), as positive controls. Two wk after minipump placement, the rats were lesioned by unilateral administration of 6-hydroxydopamine into the medial forebrain bundle. Lesioning alone decreased contralateral forelimb use and adjusted stepping, two measures of parkinsonism. ABT-107 and nicotine treatment significantly improved these behaviors at all wk tested, with variable improvements with DMXB. We next investigated the mechanism that may be involved. The striatal dopamine transporter (DAT), a marker of dopaminergic integrity, was reduced ~70% with lesioning. ABT-107, DMXB or nicotine treatment led to significantly higher DAT levels in lesioned striatum. The drugs did not alter DAT levels in intact striatum. ABT-107 and nicotine also significantly improved basal dopamine release from lesioned striatum, as well as nicotine-stimulated dopamine release mediated via α4β2* and α6β2* nAChRs. These data suggest that drugs targeting α7 nAChRs could be useful for neuroprotection in Parkinson's disease.

Disclosures: T. Bordia: None. **M. McGregor:** None. **R.L. Papke:** None. **M.J. McIntosh:** None. **M.W. Decker:** None. **M. Quik:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA030396

NIH Grant DA028955

NIH Grant NS034407

NARSAD Young Investigator Award

Title: Differential localization and function of nicotinic acetylcholine receptors in subdivisions of medial habenula

Authors: *P. SHIH[1], S. ENGLE[1], G. OH[1], P. DESHPANDE[2], N. PUSKAR[3], H. LESTER[2], R. DRENAN[1]
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) in the medial habenula (MHb) to interpeduncular nucleus (IPN) pathway are key mediators of nicotine's aversive properties. However, no previous work has systematically examined the anatomical localization and function of distinct nAChR subtypes in these two small brain regions. To address this issue, a new group of knock-in mice were created that each express a single nAChR subunit fused to green fluorescent protein (GFP), allowing high-resolution mapping. We find that α3 and β4 nAChR subunits levels are strong throughout the ventral MHb (MHbV). In contrast, α6, β2, β3, and α4 subunits are selectively found in some, but not all, areas of MHbV. All subunits were found in both choline acetyltransferase (ChAT)-positive and ChAT-negative cells in MHbV. Next, we examined functional properties of neurons in the lateral and central part of MHbV (MHbVL and MHbVC) using brain slice patch clamp recordings. MHbVL neurons were more excitable than MHbVC neurons, and they also responded more strongly to puffs of nicotine. In addition, our studies of firing responses in MHbV neurons showed that MHbVL neurons, but not MHbVC neurons, increased their firing substantially in response to a nicotine concentration (1 μM) achievable in smokers. This effect was eliminated in α4 subunit knockout mice. Bath application of SR16584, a selective antagonist for α3β4* nAChRs (the asterisk indicates the possible presence of additional subunits), also significantly blunted the ability of MHbVL neurons in response to 1 μM nicotine. These data highlight the role of α3β4* and α4* nAChRs in MHbVL neuron firing. Additionally, the increase in firing frequency by nicotine was significantly reduced in MHbVL neurons from mice that underwent withdrawal from chronic nicotine compared to mice withdrawn from chronic saline. Last, we characterized two electrophysiologically distinct neuronal populations in the dorsal part of the IPN, which receives input from MHbVL axons. Type II dorsal IPN neurons, compared to type I neurons, couple more strongly to cholinergic/glutamatergic afferents from MHbVL. Together, our data provide novel details regarding neurophysiology and nAChR localization and function in cells within the MHb-IPN tract. These results also highlight the importance of studying subregions of the MHbV to advancing our understanding of nicotine withdrawal.

Disclosures: P. Shih: None. **S. Engle:** None. **G. Oh:** None. **P. Deshpande:** None. **N. Puskar:** None. **H. Lester:** None. **R. Drenan:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

Support: Atlantic Innovation Fund

Title: Interactions between NMDA and nicotinic receptor ligands in organotypic hippocampal slice cultures

Authors: *D. HAPP[1], D. S. MACDONALD[1], H.-P. DEIGNER[2], R. A. TASKER[1]
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Abstract: NMDA receptors have been extensively characterized for their involvement in hippocampal dependent learning and memory as well as excitotoxic cell death at higher concentrations. More recently there has been considerable interest in the role of the alpha7 nicotinic receptor (a7nAChR) in hippocampal and cortical processes underlying cognition and cognitive enhancement. Several recent publications have described formation of an NMDA-a7nAChR complex (Li et al. 2012, 2013), the functional significance of which is largely unknown. To investigate the interaction between NMDA and nicotinergic-based function *in vitro* we chose to use organotypic hippocampal slice cultures prepared from P5/6 day old SD rats and grown for 14 days on 0.4 um porous membrane inserts at 37°C. Viable slices were exposed for 24 hours to varying concentrations of NMDA and re-evaluated by propridium iodide exclusion to construct toxicity-response curves. Subsequent experiments are evaluating the effect of the cholinergic agonists choline and galantamine on NMDA receptor-mediated excitotoxicity, as well as the effect(s) of corresponding antagonists. Preliminary results to date support an interaction between NMDA and nicotinergic signaling systems *in vitro*.

Disclosures: D. Happ: None. **D.S. MacDonald:** None. **H. Deigner:** None. **R.A. Tasker:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIEHS Intramural Research Program/NIH

Title: Activation of α7 nicotinic acetylcholine receptors increased intracellular cAMP levels in cultured hippocampal neurons

Authors: *Q. CHENG, J. L. YAKEL
Lab. of Neurobio., NIEHS, Durham, NC

Abstract: The activation of α7 nicotinic acetylcholine receptors (nAChRs) has been shown to improve hippocampal-dependent learning and memory. However, the molecular mechanism of α7 nAChRs' action remains elusive. We previously reported that activation of α7 nAChRs induced a prolonged enhancement of glutamatergic synaptic transmission in a protein kinase A (PKA)-dependent manner. Here, we investigated if there is a direct link between the activation of the α7 nAChR and cyclic adenosine monophosphate (cAMP) signaling in hippocampal neurons. To address this question, we employed a Förster- Resonance Energy Transfer (FRET)-based biosensor (mTurquoise-Epac(CD, ΔDEP)-^{cp173}Venus-Venus) to measure the intracellular cAMP levels directly via live cell imaging. We found that application of the α7 nAChR-selective agonist choline (2 mM; in the presence of the α7 nAChR positive allosteric modulator PNU-120596 (5 μM)) induced a significant change in the YFP/CFP ratio, which indicated an increase in intracellular cAMP levels. This choline-induced increase was abolished by the α7 nAChR antagonist MLA (40 nM) and the calcium chelator BAPTA (10 mM), suggesting that the cAMP increase depends on the α7 nAChR activation and subsequent intracellular calcium rise. The soluble adenylyl cyclase (AC) inhibitor KH7 (25 μM) also blocked the choline-induced cAMP increase, suggesting that calcium dependent ACs are required for choline's action. To determine the involvement of AC1, we tested the effect of siRNA against AC1. We found that this treatment reduced significantly the choline-induced FRET ratio change. This suggested that AC1 is the main mediator for the choline-induced cAMP rise. Our findings provide the first direct evidence to link activation of α7 nAChRs to a cAMP rise, which defines a new signaling pathway employed by α7 nAChRs. Our study sheds light into the molecular mechanisms of the positive cognitive actions of α7 nAChR agonists and development of therapeutic treatments for cognitive impairments.

Disclosures: Q. Cheng: None. **J.L. Yakel:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Program#/Poster#: 401.21/C62

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM57481

Title: Nicotinic receptors regulate inflammatory and apoptotic signaling through nonconductive states

Authors: *T. M. GOULD[1], C. W. KINTER[2], N. A. HORENSTEIN[1], R. L. PAPKE[2]
[1]Chem., [2]Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Nicotinic acetylcholine receptors (nAChRs) are present in the blood and epithelium. Paradoxically, leukocyte nAChRs remain to be proven as having conductive ion channels, yet these nAChRs regulate inflammatory and immune-mediated signaling responses to various pathogens and stimuli. Therefore, we hypothesize that nAChRs impact intracellular inflammatory signaling in leukocytes through nonconductive conformational states. We found that in the absence of detectable ion currents, nAChRs regulate inflammatory signal transduction events in three types of leukocytes: lymphocytes, monocytes and macrophages. Moreover, we confirmed that the most effective attenuators of inflammatory signaling are not prototypical ion channel agonists such as nicotine or acetylcholine, but rather ligands that stabilize non-conducting or desensitized states. As previously reported for microglia, the partial α7 nAChR agonist GTS-21 and silent agonist NS6740 significantly attenuate stimulus-induced NF-κB inflammatory signaling in both Jurkat T lymphocytes and THP-1 monocytes; these ligands are also effective suppressors of TNFα levels in LPS-stimulated RAW 264.7 macrophages. The inhibition of inflammatory signals in leukocytes by GTS-21 and NS6740 are coincident with decreased cell viability (mitochondrial respiration) as evidenced by reduced NADH dependent dehydrogenase activity. The reduction of TNFα levels in LPS-stimulated macrophages by GTS-21 also correlates with increased caspase activity. Interestingly, NS6740 potentiates LPS-stimulated NF-κB activation in monocytes while suppressing TNFα levels in LPS-stimulated macrophages. This suggests NS6740 has the ability to induce multiple nonconductive signaling modes, and that nAChRS may regulate intracellular signaling mechanisms at both the transcriptional and post-transcriptional level. NF-κB plays a crucial role in cell viability, regulating inflammatory, proliferative and apoptotic signals. Consistent with these findings, it is possible that nAChRs may induce apoptosis by inhibiting NF-κB in the attenuation of inflammation. Alternatively, diminished NF-κB signaling may be a consequence of induced

apoptosis. Our data support the hypothesis that ionotropically silent nAChRs may be metabotropically active and can regulate signaling and transcription. Our studies signify why the putatively nonconductive leukocyte nAChRs represent a major therapeutic target for the pharmacological control of inflammation and the regulation of aberrant NF-κB signaling in immune-mediated diseases and cancer, and recommend the further development of silent agonists for these indications.

Disclosures: **T.M. Gould:** 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.; GM57481. **C.W. Kinter:** A. Employment/Salary (full or part-time):; University of Florida. 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.; GM57481. **N.A. Horenstein:** A. Employment/Salary (full or part-time):; University of Florida. 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.; GM57481. **R.L. Papke:** A. Employment/Salary (full or part-time):; University of Florida. 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.; GM57481.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

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Title: Nicotine increases GABAergic input to 5-HT dorsal raphe neurons by stimulation of presynaptic α7 nicotinic acetylcholine receptors

Authors: F. HERNANDEZ VAZQUEZ[1], K. CHAVARRIA[2], J. GARDUÑO[2], S. HERNANDEZ LOPEZ[2], *S. P. MIHAILESCU[2]
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Abstract: The dorsal raphe nucleus (DRN) contains the largest populations of serotonergic (5-HT) neurons. Excitatory and inhibitory afferents from many brain areas regulates 5-HT activity. 5-HT DRN neurons express functional nicotinic acetylcholine receptors (nAChRs) and stimulation of these receptors by nicotine causes and increase in 5-HT release and 5-HT DRN neuron discharge rate. Serotonergic neurons are also excited by nicotine through increases in glutamate and noradrenaline release. However, the role of nicotine on the GABAergic input to 5-HT DRN neurons was not investigated in detail. Therefore, the aim of this work was to determine the effect of nicotine on GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) of 5-HT DRN neurons and the subtype of nAChR(s) involved in this response. Experiments were performed in coronal slices obtained from young Wistar rats. GABAergic sIPSCs were recorded in 5-HT DRN neurons using the whole-cell voltage patch clamp technique. Immunohistochemistry identification of 5-HT neurons was made after recording. The administration of nicotine (1 μM) increased sIPSCs frequency in 72% 5-HT DRN neurons. This effect was not reproduced by the α4β2 nAChR agonist RJR-2403 and was not influenced by TTX (1 μM). It was, however, mimicked by the selective agonist for α7 nAChR, PNU-282987, and exacerbated by the positive allosteric modulator of the same receptor, PNU-120596. The nicotine-induced increase in sIPSCs frequency was independent on voltage-gated calcium channels but dependent on Ca2+-induced Ca2+ release (CICR). These results demonstrate that nicotine increases the GABAergic input to most 5-HT DRN neurons, by activating presynaptic α7 nAChRs and producing CICR in DRN GABAergic terminals.

Disclosures: F. Hernandez Vazquez: None. **S.P. Mihailescu:** None. **K. Chavarria:** None. **J. Garduño:** None. **S. Hernandez Lopez:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: 2 R44 DA033744-02

Title: The effect of AT-1001, a selective α3β4 nAChR functional antagonist, on nicotine withdrawal and stress+cue induced reinstatement in rats

Authors: *M. YUAN[1], A. M. MALAGON[1], D. YASUDA[2], J. D. BELLUZZI[1], F. M. LESLIE[1], N. T. ZAVERI[2]
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Abstract: Tobacco is the leading cause of preventable death worldwide. Relapse is common during initial onset of withdrawal. Stress and smoking-related cues are also key factors potentiating relapse. Therefore, management of withdrawal and craving is critical for smoking cessation. Currently, the most effective smoking cessation aid, varenicline, only has a 23% abstinence rate after one year. Varenicline is a partial agonist at α4β2 nicotinic acetylcholine receptors (nAChR), the subtype considered predominantly responsible for smoking addiction. However, the α3β4 nAChR has recently been implicated in tobacco dependence. In addition, AT-1001, a potent and selective α3β4 nAChR functional antagonist, reduces nicotine self-administration in rats (Toll et al., 2012; Costello et al., 2014). The β4 subunit is necessary for withdrawal since mecamylamine-precipitated nicotine withdrawal is diminished in β4, but not β2, null mice. The present study tests the effect of AT-1001 on (1) precipitated nicotine withdrawal and (2) stress+cue induced reinstatement of nicotine self-administration in rats. To evaluate withdrawal, adult male Sprague-Dawley rats were exposed to chronic nicotine via subcutaneous minipumps (3.15 mg/kg/day nicotine free-base). After 7 days, rats were administered either AT-1001 (3 mg/kg, s.c.), mecamylamine (1mg/kg, s.c.), or vehicle, and nicotine abstinence signs were scored for 30 minutes. A second group was pretreated with AT-1001 or vehicle prior to mecamylamine-precipitated withdrawal, and nicotine abstinence signs were scored for 30 minutes. To examine stress+cue induced reinstatement, rats were trained to lever press for food and then allowed to self-administer nicotine (30 μg/kg/inj) at FR5. After reaching stable baseline, responding was extinguished until extinction criteria were met. Rats were then primed to reinstate nicotine-seeking behavior with yohimbine (2.5 mg/kg, i.p), a pharmacological stressor, and cues. Prior to reinstatement, one group of rats was pretreated with 0, 0.75, 1.5 or 3 mg/kg (s.c.) AT-1001 in a randomized within-subjects design. A second group was pretreated with 0, 0.5, 1, or 2 mg/kg (s.c.) mecamylamine. AT-1001 dose-dependently attenuated reinstatement of nicotine self-administration, similar to mecamylamine. However, mecamylamine produced significantly more severe withdrawal symptoms compared to AT-1001. AT-1001 pretreatment had no significant effect on mecamylamine-precipitated withdrawal. Our results suggest that AT-1001 specifically attenuates nicotine-seeking behavior, does not exacerbate nicotine withdrawal, and may be a promising pharmacotherapy for tobacco dependence.

Disclosures: M. Yuan: None. **A.M. Malagon:** None. **D. Yasuda:** None. **J.D. Belluzzi:** None. **F.M. Leslie:** None. **N.T. Zaveri:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant F31DA034490

NIDA Grant R21DA036041

Title: Upregulation of nicotinic acetylcholine receptors containing the alpha4 subunit in VTA GABAergic neurons underlies reward sensitization

Authors: *J. NGOLAB, L. LIU, P. D. GARDNER, A. R. TAPPER
Brudnick Neuropsychiatric Res. Institute, Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Nicotine is the psychoactive component in tobacco and an agonist for nicotinic acetylcholine receptors (nAChR), ligand-gated cation channels normally activated by the endogenous neurotransmitter, acetylcholine. Unlike other drugs of abuse, chronic nicotine use leads to increased expression ("upregulation") of nAChRs in the mesocorticolimbic reward pathway in addition to other brain regions. While upregulation of nAChRs is a hallmark of chronic nicotine exposure, and was first identified over 20 years ago, the behavioral consequence of this phenomenon and how it relates to nicotine dependence is unknown. Furthermore, functional upregulation of nAChRs containing the [[Unsupported Character - Symbol Font ]]4 subunit ([[Unsupported Character - Symbol Font ]]4* nAChRs) in the mesolimbic pathway has been shown to be restricted to GABAergic neurons within the VTA. However, how functional upregulation of [[Unsupported Character - Symbol Font ]]4* nAChRs affects the micro-circuitry of the VTA and nicotine dependence-related behaviors is unknown. To address this question, we engineered a Cre recombinase-dependent gene expression system to selectively express [[Unsupported Character - Symbol Font ]]4 nAChR subunits harboring a "gain of function" mutation (a leucine mutated to a serine residue at the 9' position: L9'S), that renders nAChR containing the subunit hypersensitive to nicotine, in VTA GABAergic neurons of adult male mice, essentially mimicking functional upregulation. In mice expressing L9'S [[Unsupported Character - Symbol Font ]]4 nAChR subunits in VTA GABAergic neurons ($Gad2^{VTA}$:L9'S mice), an acute subcutaneous injection of 0.09 mg/kg nicotine was sufficient to activate VTA GABAergic neurons; whereas control mice required a much higher nicotine dose, 0.5 mg/kg, to activate GABAergic neurons. To test the hypothesis that functional upregulation of [[Unsupported Character - Symbol Font ]]4* nAChRs

in VTA GABAergic neurons modulated nicotine reward sensitivity, we measured nicotine reward in $Gad2^{VTA}$:L9'S and control animals using the conditioned place preference assay. Interestingly, the sub-reward threshold dose of 0.09 mg/kg nicotine conditioned a significant place preference in $Gad2^{VTA}$: L9'S mice but not in control animals. Together, these data indicate that upregulation of [[Unsupported Character - Symbol Font ]]4* nAChRs in VTA GABAergic neurons mediate reward sensitization, a neuroadaptation that could facilitate relapse in abstinent smokers.

Disclosures: J. Ngolab: None. **L. Liu:** None. **P.D. Gardner:** None. **A.R. Tapper:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

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Topic: B.02. Ligand-Gated Ion Channels

Support: Atlantic Innovation Fund

University of Prince Edward Island

Title: Dose-dependent effects of domoic acid on expression of alpha 7 nicotinic receptors in organotypic hippocampal slice cultures

Authors: *J. R. HAWKINS, D. S. MACDONALD, R. A. TASKER
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Abstract: Recently the alpha7 nicotinic receptor (a7nAChR) has been shown to be involved in various forms of cognition and is a promising target for cognition enhancing drugs. At high concentrations, the marine toxin, domoic acid (DOM), is known to produce hippocampal cellular toxicity primarily by an AMPA-kainate receptor-dependent mechanism (Verdoorn et al. 2004; Tasker et al. 2006). However, at sub-toxic concentrations DOM induces both acute (Tasker et al. 2005) and persistent (Gill et al. 2012) deficits in cognition when administered to neonatal rats. To determine if DOM-induced deficits in cognition might be attributable, at least in part, to an effect on a7nAChRs we investigated the dose-dependent effects of DOM on cell-specific viability (propridium iodide exclusion) and a7nAChR expression (Western blot) *in vitro* using organotypic hippocampal slice cultures (OHSCs). Cultures were prepared from P5/6 day old SD rats and grown for 14 days on 0.4 um porous membrane inserts at 37°C. Viable slices were

exposed for 24 hours to varying concentrations of DOM and re-evaluated by propridium iodide exclusion to construct toxicity-response curves. The effects of sub-toxic concentrations of DOM on the expression of a7nAChRs was measured using Western blot analysis of pooled slice cultures normalized against total protein concentration. Preliminary results indicate that DOM reduces a7nAChR expression in a dose-dependent manner in OHSCs.

Disclosures: J.R. Hawkins: None. **D.S. MacDonald:** None. **R.A. Tasker:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.26/C67

Topic: B.02. Ligand-Gated Ion Channels

Support: UAE university Grant

Title: Apigenin potentiates human α7-nicotinic acetylcholine receptors

Authors: *K.-H. S. YANG[1], T. R. PRYTKOVA[1], S. M. NURULAIN[2], F. C. HOWARTH[3], M. OZ[2]
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Abstract: Flavonoids are a group of small molecules derived from plant-based compounds. They have received considerable attention for their therapeutic use against cancer, heart disease and other pathological conditions. Apigenin (4,5,7-trihydroxyflavone) is one of the most widely studied dietery flavonoid found in different plants. Extracts of apigenin have been shown to have sedative, anxiolytic, and antidepressant actions. However, molecular and cellular mechanisms of apigenin actions in the central nervous system currently remain unknown. In the present study, effects of apigenin on the function of the cloned α7 subunit of human nAChR expressed in Xenopus oocytes were investigated using the two-electrode voltage-clamp technique. Apigenin, in the concentrations of 10 and 30 μM potentiated of nAChR-mediated currents (induced by 100 μM ACh) to 40 % and 60 % of controls, respectively. Potentiation by apigenin was reversible and concentration dependent. In conclusion, the results indicate that apigenin potentiates the function of human α7-nAChRs.

Disclosures: **K.S. Yang:** None. **T.R. Prytkova:** None. **S.M. Nurulain:** None. **F.C. Howarth:** None. **M. Oz:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.27/C68

Topic: B.02. Ligand-Gated Ion Channels

Title: Inhibition of nicotinic acetylcholine receptors by philanthotoxins is strongly influenced by subunit composition

Authors: ***H. KACHEL**[1], H. FRANZYK[2], K. STRØMGAARD[2], D. TIKHONOV[3], I. MELLOR[1]
[1]Univ. of Nottingham, NOTTINGHAM, United Kingdom; [2]Dept. of Mol. Drug Res., Univ. of Copenhagen, Copenhagen, Denmark; [3]Sechenov Inst. of Evolutionary Physiol. and Biochem., Russian Acad. of Sci., St. Petersburg, Russian Federation

Abstract: Abstract Philanthotoxin-433 (PhTX-433) is active component of the Egyptian solitary digger wasp, *Philanthus triangulum*, venom which non-selectively inhibits several excitatory ion channels. To improve selectivity two synthetic analogues, Philanthotoxin-343 (PhTX-343) and Philanthotoxin-12 (PhTX-12), were developed. Previous work by Brier et al. (2003) showed a 22-fold better selectivity for PhTX-12 on embryonic muscle-type nicotinic acetylcholine receptors (M-nAChR) naturally expressed in the TE671 cell line in comparison to PhTX-343. In this study, we investigated the pharmacological action of both analogues on mammalian hetero- and homooligomeric neuronal nicotinic acetylcholine receptor (N-nAChR) subunit combinations expressed in Xenopus oocytes. Whole-cell currents in response to application of acetylcholine alone or co-applied with PhTX-analogue were studied electrophysiologically using two-electrode voltage-clamp at three different membrane holding potentials (V_H = -60 mV, -80 mV and -100 mV). Concentration-inhibition curves were constructed and IC_{50} values estimated for each holding potential. The IC_{50} values for PhTX-343 inhibition of α3β4, α3β2, α4β2, α4β4 and α7 peak currents at -100 mV were 0.077 μM (n=9), 3.20 μM (n=8), 0.170 μM (n=7), 0.28 μM (n=6) and 8.7 μM (n =9) respectively; for PhTX-12 they were 2.03 μM (n=8), 36.0 μM (n=10), 0.430 μM (n=7), 2.7 μM (n=8) and 12.1 μM (n =10) respectively; i.e. in contrast to M-nAChR, PhTX-343 was more potent than PhTX-12 in all cases. The variation in potency is most likely due to a single amino acid change in the β2/4 subunit pore lining region. For inhibition of heteromeric N-

nAChRs, the potency of PhTX-343 was strongly augmented by holding the cell at more negative V_H while this was not the case for PhTX-12 where only weak voltage-dependence was observed. The inhibition of homomeric α7 receptors by both toxins was voltage-independent. Therefore, we conclude that PhTX-343 works as a potent open channel blocker of the mammalian heteromeric N-nAChR and has binding site deep in the channel while the PhTX-12 site is near to the outside of channel. In contrast, inhibition of homomeric α7 receptors may be through interaction at an alternative site outside the channel pore. Brier, T.J., Mellor, I.R., Tikhonov, D.B., Neagoe, I., Shao, Z., Brierley, M.J., Stromgaard, K., Jaroszewski, J.W., Krogsgaard-Larsen, P., Usherwood, P.N., 2003. Contrasting actions of philanthotoxin-343 and philanthotoxin-(12) on human muscle nicotinic acetylcholine receptors. Molecular pharmacology 64, 954-964.

Disclosures: H. Kachel: None. **H. Franzyk:** None. **K. Strømgaard:** None. **D. Tikhonov:** None. **I. Mellor:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.28/C69

Topic: B.02. Ligand-Gated Ion Channels

Title: Effects of Phantasmidine on neuronal nicotinic ACh and serotonin 5-HT3 receptors

Authors: *A. A. PANDYA[1], J. YAKEL[2]
[1]Univ. of Alaska Fairbanks, KOTZEBUE, AK; [2]Lab. of Neurobio., Natl. Inst. of Envrn. Hlth. Sciences, Natl. Inst. of Hlth., Research Triangle Park, NC

Abstract: Phantasmidine is a condensed tetracyclic alkaloid obtained from the Ecuadorian poison frog Epipedobates anthonyi. We tested the functional effects of synthetic phantasmidine on various subtypes of neuronal nicotinic ACh receptors (nAChRs; α7, α4β2 and α3β2) and the serotonin 5-HT3 receptors (5-HT3ARs) that had been expressed in Xenopus oocytes using two-electrode voltage-clamp techniques. We found that phantasmidine is a full agonist for the α7 receptors (with EC50 values of 9.89 + 0.90 μM), and a partial agonist for the α3β2 and α4β2 nAChRs (with an EC50 value of 19.0 + 13 μM and 3.06 + 0.19 nM, respectively). The relative peak amplitudes of responses induced by phantasmidine (compared to 1 mM ACh) is 1.08 + 0.04, 0.32 + 0.04, and 0.25 + 0.08 for α7, α4β2 and α3β2 nAChRs, respectively. For the 5-HT3ARs, phantasmidine has a lower potency than that seen with any of the nAChRs (an EC50

value of 29.1 + 1.7 μM with a relative peak amplitude of 0.79 + 0.05 compared to 10 μM mCPBG). The phantasmidine-induced currents for the α7 and α4β2 subtype of nAChRs, as well as the 5-HT3ARs, were completely blocked by methyllycaconitine (30 nM), dihydro-β-erythroidine (10 nM), and tropisetron (30 nM), respectively. This shows that the functional effects of phantasmidine are due to its binding to the agonist site on these receptors since competitive antagonists are able to inhibit it's actions. In summary, phantasmidine is an agonist for α7, α4β2 and α3β2 nAChRs and the 5-HT3ARs with the potential for clinical applications.

Disclosures: **A.A. Pandya:** A. Employment/Salary (full or part-time):; University of Alaska Fairbanks, Department of Biomedical Sciences, College of Rural and Community Development. **J. Yakel:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 401.29/C70

Topic: B.02. Ligand-Gated Ion Channels

Support: Wings for Life Spinal Cord Research Grant

Title: Cytoskeletal motility and structural growth in developing neural cells is driven by α7-nicotinic acetylcholine receptor association with G proteins

Authors: J. R. KING[1], J. C. NORDMAN[1], S. P. BRIDGES[1], S. BLACK[1], D. P. VELTRI[1], M. LIN[1], A. SHEHU[1], *N. KABBANI[2]
[1]George Mason Univ., Fairfax, VA; [2]Mol. Neurosci, Krasnow Inst., FAIRFAX, VA

Abstract: Several heterotrimeric G proteins are enriched at sites of cellular growth and can regulate the cytoskeleton during development and structural plasticity. We have recently shown a role for the α7 nicotinic acetylcholine receptor (α7 nAChR) in the regulation of axon growth in hippocampal neurons via the binding of a G protein signaling complex. Here we have generated a series of α7 nAChR mutants and characterized their ability to bind and signal via G proteins in developing neural cells. Immunoprecipitation studies suggest that a series of basic amino acid residues within the M3-M4 loop of the nAChR enable G protein coupling. Interactions between α7 nAChRs and G proteins were examined in the adult and developing rodent brain, and within synaptic and non-synaptic compartments. Cellular imaging studies reveal that activation of the α7 nAChR/G protein pathway directs cytoskeletal motility in neurites and growth cones.

Interaction with G proteins thus appears to contribute to changes in cholinergic modulation of axon growth and branching during brain development. Based on these findings we propose the existence of a G protein binding site within nAChRs. This domain appears to have evolved to augment the signaling and regulatory properties of this ligand gated ion channel in cells.

Disclosures: J.R. King: None. **N. Kabbani:** None. **J.C. Nordman:** None. **S.P. Bridges:** None. **S. Black:** None. **D.P. Veltri:** None. **M. Lin:** None. **A. Shehu:** None.

Poster

401. Nicotinic Receptors: Physiology and Pharmacology

Location: Halls A-C

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Program#/Poster#: 401.30/C71

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Intramural Clinical and Biological Research Z1A AA000466

Title: Varenicline attenuates ventromedial prefrontal cortex activity, modulates reward circuitry for alcohol in heavy drinkers

Authors: *J. L. GOWIN, V. VATSALYA, R. MOMENAN, V. A. RAMCHANDANI
LCTS, Natl. Inst. On Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Nearly 4% of deaths worldwide are attributed to alcohol, making it a major public health burden. Recent clinical trials have shown that varenicline, a α4β2 nicotinic partial agonist used for smoking cessation, can reduce heavy drinking among alcoholics. It remains unclear, however, what neural and cognitive mechanisms are affected by varenicline to reduce drinking. Previous studies have shown that the striatum and ventromedial prefrontal cortex (vmPFC) contribute to evaluation of outcomes. Striatal activation may indicate the value of a reward, and the vmPFC may integrate value and cost. Evaluation may involve a circuit beginning in the ventral striatum, then moving to the dorsal striatum before signaling the vmPFC. No study has examined how varenicline may affect this reward circuit in heavy drinkers. Here, we studied heavy drinkers given either varenicline (2mg/day) or placebo for 2 weeks prior to a functional MRI scan. During the scan, participants completed an incentive task where they had the opportunity to earn food, alcohol or neutral (no gain or loss) rewards. They saw cues for the rewards and then had to press a button during a brief response window. A button press within the response window resulted in earning points for the reward, while a response outside the window resulted in a missed reward. 30 heavy drinkers (5 female) were scanned. The placebo group

(N=13) showed increased vmPFC activation when missing a drink reward relative to a neutral or food reward, while the varenicline group (N=17) showed roughly equivalent activation when missing drink, food and neutral points. Direct contrast of treatment groups indicated that the varenicline group showed significantly decreased vmPFC activation relative to the placebo group when missing a drink reward. Functional connectivity analysis across the entire time series focusing on a vmPFC seed indicated significant connectivity with the dorsal striatum in the placebo group, with no significant effects in the varenicline group. Strength of connectivity between vmPFC and striatum showed a trend toward a positive relationship with vmPFC activation when missing drink points, where individuals with higher connectivity had higher activation. These results indicate that varenicline may decrease the value of alcohol rewards in the vmPFC, and this decrease may be related to disrupted functional circuitry with the striatum in heavy drinkers.

Disclosures: J.L. Gowin: None. **V. Vatsalya:** None. **R. Momenan:** None. **V.A. Ramchandani:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 402.01/C72

Topic: B.06. Neurotransmitter Release

Support: NSC

Title: Synapsin Ia, a synaptic vesicle protein, regulates the dynamics of dense-core vesicle exocytosis

Authors: *H.-J. YANG, Y.-C. CHANG, C.-T. WANG
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Abstract: Neurotransmitters are packaged into two distinct classes of vesicles, synaptic vesicles (SVs) and dense-core vesicles (DCVs). Although the release from both vesicle classes shares a common mechanism of Ca2+-dependent exocytosis, a particular phosphoprotein, synapsin (Syn), localizes to SVs exclusively. The Syn protein family consists of ten homologous proteins, of which Syn Ia is the best studied. Upon phosphorylation by various protein kinases, Syn Ia can recruit SVs to plasma membrane, thereby increasing the dynamics of SV exocytosis. However, it remains unknown whether SV recruitment to plasma membrane may also facilitate the dynamics

of DCV exocytosis. Our preliminary results showed that in the rat hypothalamic-neurohypophysial system (HNS), Syn Ia increased oxytocin release by phosphorylation at the serine 62 site (Ser-62), suggesting that that Syn Ia phosphorylation may facilitate the release from DCVs. To further determine the Syn Ia's regulation of DCV exocytosis at the single-vesicle level, we directly measured norepinephrine release from DCVs by performing single-vesicle amperometry in PC12 cells. The foot signal preceding amperometric spikes (prespike foot, PSF) has been shown to represent the fusion pore dynamics. Secretory events were triggered by direct application of high extracellular KCl onto the cells. We found that Syn Ia significantly increased the secretion rate by phosphorylation at multiple serine sites (Ser-9, Ser-566, and Ser-603). These results suggested that Syn Ia may facilitate the secretion rate of DCVs by CaMK phosphorylation. In contrast, Syn Ia significantly increased the PSF lifetime by phosphorylation at Ser-62, suggesting that Syn Ia may stabilize fusion pores by MAPK phosphorylation. Immunostainings confirmed that Syn Ia did not localize to the DCVs in PC12 cells. Thus, our results suggest that the SV-specific protein Syn Ia may play an important role in regulating the dynamics of DCV exocytosis.

Disclosures: H. Yang: None. **Y. Chang:** None. **C. Wang:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

Location: Halls A-C

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Program#/Poster#: 402.02/D1

Topic: B.06. Neurotransmitter Release

Support: NIH grant T32 DA07274 (J.P.B.)

NIH grant GM 58055 (H.C.H.)

Title: Anesthetic effects on the calcium sensitivity of synaptic vesicle exocytosis in hippocampal neurons

Authors: *J. P. BAUMGART[1,3], Z. ZHOU[1], M. HARA[1], M. B. HOPPA[2,4], T. A. RYAN[2], H. C. HEMMINGS[1]
[1]Anesthesiol., [2]Biochem., Cornell Univ. Weill Med. Col., New York, NY; [3]Natl. Inst. of Hlth., Bethesda, MD; [4]Biol., Dartmouth Col., Hanover, NH

Abstract: The molecular and cellular mechanisms of anesthetic-induced amnesia, unconsciousness and immobilization are poorly understood. Identification of presynaptic mechanisms is critical to understanding the effects of anesthetics on synaptic transmission. Here we used quantitative imaging methods to show that the prototypical volatile anesthetic isoflurane inhibits synaptic vesicle exocytosis in cultured rat hippocampal neurons proportional to reductions in presynaptic [Ca^{2+}]. This finding indicates that isoflurane reduces [Ca^{2+}]$_i$ without altering the Ca^{2+} sensitivity of synaptic vesicle exocytosis. Greater inhibition by isoflurane of exocytosis in glutamatergic compared to GABAergic neurons can be explained by larger reduction in intracellular [Ca^{2+}], which correlated with inhibition of exocytosis. These data suggest that volatile anesthetic inhibition of neurotransmitter release from small synaptic vesicles involves primarily targets that reduce Ca^{2+} entry without significant direct effects on the synaptic vesicle fusion machinery.

Disclosures: J.P. Baumgart: None. **Z. Zhou:** None. **M. Hara:** None. **M.B. Hoppa:** None. **T.A. Ryan:** None. **H.C. Hemmings:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Program#/Poster#: 402.03/D2

Topic: B.06. Neurotransmitter Release

Support: FWF grant P 24909-B24

Title: Nanodomain coupling between Ca2+ channels and release sensors explains the apparent Ca2+ independence of transmitter release time course at a GABAergic synapse

Authors: I. ARAI, *P. JONAS
IST Austria, Klosterneuburg, Austria

Abstract: A highly puzzling property of synaptic transmission is that the time course of transmitter release is independent of extracellular Ca^{2+} concentration ([Ca^{2+}]$_o$), whereas the rate of release is highly [Ca^{2+}]$_o$-dependent. However, it is unclear whether this apparent discrepancy, originally observed at the neuromuscular junction, also applies to central synapses. Furthermore, the underlying mechanistic explanation remains elusive. To address these questions, we measured the time course of release at GABAergic synapses between basket cells (BCs) and Purkinje cells (PCs), using paired whole-cell recordings from synaptically connected neurons in

mouse cerebellar slices at ~22°C. The release time course was obtained by deconvolution. When $[Ca^{2+}]_o$ was changed from 2 mM to either 1 mM or 4 mM, peak transmitter release rate was markedly altered. In contrast, the release time course was only minimally affected (half-duration at 2 mM: 0.45 ± 0.02 ms; 1 mM: 0.39 ± 0.03 ms; 4 mM: 0.46 ± 0.02 ms; mean ± SEM; 4–9 pairs). Thus, the time course of release at BC-PC synapses is largely $[Ca^{2+}]_o$-independent. We next examined the possible reasons for this apparent $[Ca^{2+}]_o$ independence. Recent results suggested that cerebellar BC-PC synapses show tight coupling between Ca^{2+} channels and release sensors (Christie et al., Nat. Neurosci. 14:62–68, 2011), raising the possibility that tight coupling may underlie $[Ca^{2+}]_o$ independence. To test this hypothesis, we probed the coupling distance by loading Ca^{2+} chelators with different Ca^{2+}-binding rates into the presynaptic neurons. BAPTA, a fast Ca^{2+} chelator, suppressed evoked IPSCs at submillimolar concentrations (IC_{50}: 0.6 mM, 14 pairs). In contrast, EGTA, a slow Ca^{2+} chelator, was much less effective (IC_{50}: 16.0 mM, 11 pairs). Describing the experimental data with a simple model of buffered diffusion revealed a coupling distance of ~20 nm. To further test whether tight coupling was sufficient to explain the $[Ca^{2+}]_o$ independence of release time course, we simulated release at different $[Ca^{2+}]_o$ using a realistic coupling model and a previously established release sensor model (Lou et al., Nature 435:497–501, 2005). Interestingly, models with tight coupling produced smaller Ca^{2+} dependence of release time course than those with loose coupling. Thus, tight coupling between Ca^{2+} channels and release sensors may explain the apparent Ca^{2+} independence of release time course. Constancy of time course of release might be particularly relevant at cerebellar GABAergic BC-PC synapses, helping to provide fast and precise feedforward inhibition during temporally fluctuating extracellular Ca^{2+} concentrations.

Disclosures: I. Arai: None. **P. Jonas:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 402.04/D3

Topic: B.06. Neurotransmitter Release

Title: Syntaxin1B deficiency affects Ca^{2+} sensitivity of vesicle fusogenicity and short term plasticity at the Calyx of Held

Authors: Q. GUO, J. GUO, X. WANG, *J. SUN
Chinese Acad. of Sci., Beijing, China

Abstract: Syntaxin1 (STX1), including Syntaxin1A (STX1A) and Syntaxin1B (STX1B), is the t-SNARE protein at presynaptic membrane and considered to be a key component in vesicle release. To determine whether /how STX1B functions in synaptic transmission as well as short term plasticity, a STX1B hypomorph mouse line with~5% expression of STX1B was analyzed at the calyx of Held synatpse. We found that mostly removal of STX1B significantly reduced basal synaptic transmission and enhanced synaptic depression; the deficiency of STX1B also significantly decreased readily releasable pool(RRP) size and largely slowed the vesicle priming process while Ca^{2+} channel activity was kept unchanged. Moreover, this STX1B hypomorph mutation was found to tighten, instead of weaken, the Ca^{2+} channel-vesicle spatial coupling. By flash photolysis uncaging Ca^{2+} and postsynaptic EPSC recording, we further studied the dependence of vesicle release and found that STX1B deficiency caused significant reduction of apparent Ca^{2+} sensitivity of vesicle release. Our biophysical modelling justified that reduction in the apparent Ca^{2+}-affinity of fusogenicity be due to the decrease in the number of assembled SNARE complexes per vesicle during fusion. In summary, our study shows that STX1B protein is a critical determinant in synaptic transmission and plasticity; in spite of Ca^{2+} sensor proteins, the number of assembled SNARE-complexes per vesicle plays a pivotal role in determining the intrinsic Ca^{2+} sensitivity and thus to regulate vesicle release probability as well as short term plasticity.

Disclosures: Q. Guo: None. **J. Guo:** None. **J. Sun:** None. **X. Wang:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Program#/Poster#: 402.05/D4

Topic: B.06. Neurotransmitter Release

Support: CIHR Grant

Title: The N-terminal peptide of syntaxin-1 differentially contributes to neurosecretion depending on the conformational state of the protein

Authors: *S. PARK[1,2], S.-Y. KANG[1], T.-C. CHOU[1], L. PARSAUD[1,2], S. SUGITA[1,2]
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Abstract: As neurons communicate via neurotransmitters, the release of these chemicals is a key process. During the process, fusion of chemical-storing vesicles with the plasma membrane is

believed to be executed by a complex of three SNARE (the soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins: SNAP-25, VAMP2, and syntaxin-1. Of the SNAREs, syntaxin-1 is regulated through two conformations: open and closed. When the protein adopts the open conformation, it can assemble into a complex with the other SNAREs. In the closed conformation, the protein folds in half and binds with another protein: Munc18-1. As a result of this property, the latter conformation inhibits the SNARE assembly. For the past few years, several studies have demonstrated that the association of syntaxin-1 and Munc18-1 is not limited to forming the closed conformation; the N-terminal peptide of syntaxin-1 is essential for Munc18-1 to interact with the SNARE complex and accelerate SNARE-mediated fusion. However, we hypothesized that the N-terminal peptide is critical for securing the binary interaction of syntaxin-1 and Munc18-1. To test this hypothesis, we utilized both *in vitro* and *in vivo* systems. Introduction of mutations into the N-peptide of syntaxin-1 did not affect secretion from PC12 cells, although the localization of the mutants was perturbed to some degree when visualized using confocal microscopy. By contrast, introduction of the same mutations into a syntaxin-1A mutant form favoring open conformation severely impaired secretion and localization. Since different results were yielded depending on the conformational state of syntaxin-1, we investigated by employing a GST-pulldown technique to determine whether N-terminal mutations affect the strength of the binary interaction between syntaxin-1 and Munc18-1. We found that an N-terminal mutation alone slightly weakened the binary interaction, but the same mutation additionally introduced in the open mutant strongly disrupted it. Furthermore, we tested the hypothesis using C.elegans. Unc-64 (syntaxin-1 homolog) null mutant worms that exhibit a lethal phenotype were largely rescued with mammalian syntaxin-1A wild-type, the open mutant, or some of the N-terminal mutant forms tested in the *in vitro* system. However, the double mutant that exhibited strongly disrupted binding to Munc18-1 in the GST-pulldown experiment poorly rescued the lethal phenotype. Based on the results obtained from both model systems, we conclude that the N-peptide is not essential in exocytosis despite its role in securing the binary interaction between syntaxin-1 and Munc18-1.

Disclosures: **S. Park:** None. **S. Kang:** None. **T. Chou:** None. **L. Parsaud:** None. **S. Sugita:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Program#/Poster#: 402.06/D5

Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS44057.

Title: Synaptophysin regulates fusion pores and the mode of Ca2+-triggered exocytosis of dense-core vesicles in chromaffin cells

Authors: *C.-W. CHANG[1], M. B. JACKSON[2]
[1]Physioloy Grad. Training Program, [2]Neurosci. Dept., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Synaptophysin (syp) is one of the most abundant proteins in synaptic vesicles (SVs), with ~32 copies per vesicle and accounting for ~10% of total SV protein mass. Syp has four transmembrane segments and is homologous in structure to two other proteins, synaptogyrin and synaptoporin. Syp may have a role in SV endocytosis and the retrieval of the v-SNARE protein, synaptobrevin 2. Although syp is considered SV specific and is widely used as a synaptic terminal marker, some evidence indicates its presence on large dense core vesicles (LDCVs), but at a lower density than SVs. To examine the role of syp in LDCV exocytosis, we used amperometry to record the release of catecholamine in chromaffin cells. In a comparison between cells from wild-type (WT) and syp knockout (KO) mice, the secretion rate was reduced by 30% in syp KO cells but most of the characteristics of single-LDCV full fusion events remained unchanged. The quantal size, rise time, decay time, and spike half-width of full fusion events of syp KO cells were the same as in WT. However, pre-spike feet (PSF) had shorter durations and the fraction of kiss-and-run events was higher in syp KO cells. Thus, syp may control fusion pore stability and influence the choice of LDCVs between full fusion and kiss-and-run. Transfection of syp KO cells with syp-mCherry fusion protein rescued the reduction of PSF duration and reversed the increase in kiss-and-run, but did not rescue the low secretion rate. Furthermore, syp-mCherry overexpression increased the quantal size and half-width of full fusion events. In both WT cells transfected with syp-mCherry and syp KO cells transfected with syp-mCherry we saw similar secretion, with no significant differences between PSF duration, fraction of kiss-and-run, quantal size, and half-width of full fusion events. Thus, while fusion pore stability and fate are sensitive to the absence of syp, overexpression has a greater impact on exocytosis after fusion pores start to dilate, influencing later steps of exocytosis that control quantal size and spike half-width. This implies roles for syp both on nascent fusion pores early in exocytosis and later in exocytosis during fusion pore dilation. The relatively low copy numbers of syp on LDCVs may account for the different results between KO and overexpression, and some of these results may reflect some replacement of function by synaptogyrin and synaptoporin. In summary, these results suggest that syp functions in LDCV exocytosis in chromaffin cells, controlling fusion pore stability, fate, and dilation.

Disclosures: C. Chang: None. **M.B. Jackson:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

Location: Halls A-C

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Program#/Poster#: 402.07/D6

Topic: B.06. Neurotransmitter Release

Support: NSC

Title: Synaptotagmin III modulates the kinetics of regulated exocytosis by Ca2+ binding to the C2AB domains

Authors: *Y.-T. HUANG, Y.-T. HSIAO, S.-Y. KAO, Y.-C. CHEN, C.-T. WANG
Inst. of Mol. and Cell. Biol., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Synaptotagmin (Syt) protein family consists of at least seventeen isoforms, and most of them serve as the Ca2+ sensor during regulated exocytosis. During the critical period of visual circuit development (postnatal day P6 in rats), we found that Syt III, but not Syt I, is transiently expressed in the retinal output neurons (retinal ganglion cells) and optic nerves. Previous studies showed that Syt I can modulate the kinetics of Ca2+-regulated exocytosis. However, it remains unclear whether Syt III may modulate the kinetics of Ca2+-regulated exocytosis. In this study, we investigated whether Syt III modulates regulated exocytosis and if this modulation involves Ca2+ binding to its C2AB domains. By combining molecular perturbation and single-event amperometry, the real-time exocytosis of single vesicles was detected in PC12 cells overexpressing control vector, wild-type Syt III (Syt III), or the Syt III mutant with the abolished Ca2+-binding sites at the C2AB domains (D386,520N, designated Syt III-C2AB*). We found that the secretion rate was reduced to a half in the cells overexpressing Syt III-C2AB* compared to control or Syt III. Therefore, through Ca2+ binding to the C2AB domains, Syt III may have a great capacity to modulate the kinetics of regulated exocytosis.

Disclosures: Y. Huang: None. **Y. Hsiao:** None. **S. Kao:** None. **Y. Chen:** None. **C. Wang:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

Support: NIH research grant MH64827

Title: Optogenetic-mediated glutamate release from the rat amygdala

Authors: *G. LONART, L. L. WELLMAN, M. MACHIDA, M. F. FITZPATRICK, L. D. SANFORD
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Abstract: Optogenetics allows detecting and controlling intact neural pathways with high spatio-temporal resolution, both during and after the performance of complex behaviors (Deisseroth, 2014). Recording of electrical activity is commonly used to monitor the influence of optogenetic manipulations at the cellular level. A complementary approach, measuring neurochemical release, may be especially useful when the identity of the neurotransmitter is unknown or when a rapid or post-experiment assay is desired. To explore the compatibility of optogenetic and neurochemical approaches, we infected the basolateral nucleus of the amygdala (BLA) of rats with AAV-CaMKIIa-eNpHR3.0-eYFP (eNpHR3.0) and AAV5-CaMKIIa-hChR2(H134R)-eYFP (hChR2) vectors (University of North Carolina Vector Core, Chapel Hill, NC), where expression is under the control of a calcium-calmodulin dependent kinase IIa (CaMKIIa) promoter, allowing selective targeting of a subpopulation of glutamatergic neurons. Afterwards, the transduced cells express opsins that mediate either chloride (eNpHR3.0) or cation (hChR2) influx upon activation by light, allowing hyperpolarization (inhibition) or depolarization (activation), respectively. The vectors are fused to yellow fluorescence protein to enable localization in target neurons. Channel activation was produced using blue (473 nm) or green (532 nm) light from fiber coupled lasers (Laser Century) with ~10 mW output at the fiber tip to activate cation (hChR2) or chloride (eNpHR3.0) channels, respectively. We assessed light-evoked glutamate release *in vitro* using a slice preparation labeled with 14C-glutamate. As shown in Fig. 1, stimulation with blue light produced a release of glutamate in BLA that was blunted by concurrent stimulation with green light.These findings demonstrate the compatibility of optogenetic and neurochemical methods, which may be a valuable approach for identifying neurotransmitters of neuronal circuits.*Reference: Deisseroth K (2014) Circuit dynamics of adaptive and maladaptive behaviour. Nature 505: 309-17.*

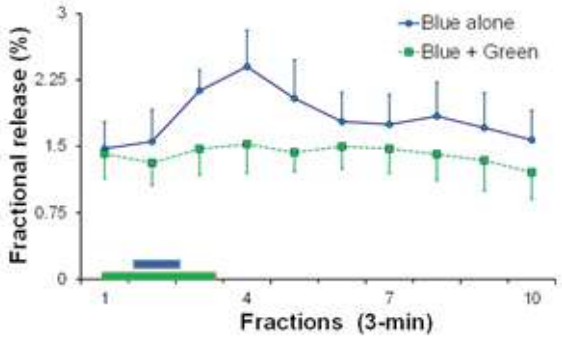

Fig. 1. Effects of laser stimulation (indicated by colored bars; blue: 3 trains of 20Hz, 30 sec, 5 ms pulse, 2 sec between trains; green: continuous) on ^{14}C-Glu release from rat amygdala slices after transduction of BLA with hChR2 and eNpHR3.0. Plots represent means across 3 stimulations in 3 rats.

Disclosures: G. Lonart: None. **L.L. Wellman:** None. **M. Machida:** None. **M.F. Fitzpatrick:** None. **L.D. Sanford:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 402.09/D8

Topic: B.06. Neurotransmitter Release

Support: PRIN Grant 2009YHXJ85_002

Title: Behavioral responses of Drosphila melanogaster lines carrying SNAP25 or Syntaxin point mutations

Authors: *A. MEGIGHIAN[1], O. ROSSETTO[1], M. SCORZETO[1], C. MONTECUCCO[1], M. A. ZORDAN[2]
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Abstract: We recently demonstrated, in *Drosophila* melanogaster, that the substitution of R in position 206 of SNAP25, or D in position 253 of Syx, with residues carrying an opposite charge,

prevents the assembly of SNARE complexes in a rosette-like supercomplex arounf the point of contact between neurotrasmitter vesicle membrane and presynaptic membrane. The formation of SNARE supercomplex plays a fundamental role in regulating neurotransmitter vesicle fusion and we have demonstrated that in our mutants evoked and spontaneous neurotrasmitter realease is impaired even if not blocked. Schizophrenia (SZ) was described since the beginning as a "sejuncto psichose", i.e. a mental disease due to a "disconnection" between different brain regions. Until now, few and contradictory signs of neuronal damage were found in patients soffering from this disease, leading to the hypothesis that the core of SZ physiopathological changes coulsd reside in a dysfunction of synapse. Among the various synaptic proteins which can be involved in the above mentioned changes, they were also considered the SNAREs. Some genetic data seem to indicate that SNAP25 levels are altered in SZ patients. Considering these hypotheses, we carried on various behavioral analyses using our mutant lines, which showed a variable and differential phenotype in each type of analysed behaviors . These data seem to indicate that the reduced synaptic strenght, observed with electrophysiological techniques, is able to induce a variety of functional impairments at the level of the complex neuronal circuits regulating different behavioral responses. These results could give support to the idea that also minor changes in synaptic function, if translated to complex neuronal circuits, could alter the flux of informations and their integration, leading to the changes in behavior and cognitive processes typical of some mental diseases lacking any clear pathological mark.

Disclosures: A. Megighian: None. **O. Rossetto:** None. **M. Scorzeto:** None. **C. Montecucco:** None. **M.A. Zordan:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Support: Instituto Salud Carlos III

FEDER

MINECO BFU2010-15713

Junta de Andalucia P12-CTS-2232

Title: Functional imaging of presynaptic calcium modulation mediated by metabotropic GABAB receptors

Authors: J. A. MARTINEZ-LOPEZ, F. MAVILLARD, L. GOMEZ-SANCHEZ, *R. FERNANDEZ-CHACON
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Abstract: Genetically-encoded fluorescent calcium sensors are very useful tools to image neural activity. Here we have used the calcium sensor GCaMP3 fused to the synaptic vesicle synaptophysin (syGCaMP3, Li et al., Front. Mol. Neurosci. 4:34, 2011) to study cytosolic calcium dynamics at the presynaptic terminals in mouse hippocampal cultures. Neurons were infected with a lentiviral vector to drive the expression of syGCaMP3 under the human synapsin promoter (Gascon et al., J Neurosci Methods 168: 104, 2008). Using epifluorescence microscopy, we have detected prominent calcium signals upon depolarization by electrical field stimulation (10 s, 20 Hz). Interestingly, attending to the duration of calcium transients we observed two clearly different types of responses: (1) long duration responses (LDR) characterized by a persistent elevation of calcium levels concomitant with the stimulus application and (2) short duration responses (SDR) characterized by a transient elevation of calcium levels of similar amplitude to LDR but with, however, shorter duration (4.94±1.35 s, mean±sem, 134 ROIs, 4 coverslips, 2 cultures; size of manually selected ROIs 1.66 squared microns). We have used correlation analysis based on the time course of responses to get spatial separation of two synaptic populations displaying either LDR or SDR (Junek et al., Biophys. J. 96:3801, 2009). The majority of synapses presented LDRs. In order to investigate the synaptic modulation underlying LDR and SDR we blocked GABAergic synaptic transmission. Incubation with the non-competitive GABAA-receptor antagonist picrotoxin did not change either the frequency of occurrence or the characteristics of LDR and SDR. In contrast, in the presence of the GABAB-receptor antagonist CGP-55845, the LDR dwell times were unchanged, while in the case of SDR, CGP-55845 extended the dwell time to coincide with the stimulus duration. Next, we combined calcium imaging with synaptic vesicle imaging. We used fluorescently-labeled antibodies against the luminal domain of the vesicular GABA transporter for the selective labeling of active GABAergic synapses (Martens et. al., J. Neurosci. 28:13125, 2008). We found that none of the synapses expressing syGCaMP3 were active GABAergic synapses, likely because the lentiviral vector preferentially labeled excitatory synapses (Nathason et al., Neuroscience 161:441, 2009). Our observations suggest that the SDR likely reflect presynaptic inhibition of glutamatergic synapses mediated by GABAB receptors. In addition, calcium imaging with syGCaMP3 opens new possibilities to study molecular mechanisms of presynaptic modulation mediated by metabotropic GABAB receptors.

Disclosures: J.A. Martinez-Lopez: None. **R. Fernandez-Chacon:** None. **F. Mavillard:** None. **L. Gomez-Sanchez:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS075506

Title: Molecular insights into the regulation of synaptic transmission by protein ubiquitination

Authors: *A. CAPUTO[1], K. M. MYERS[1], A. A. VASHISHT[2], J. A. WOHLSCHLEGEL[2], F. E. SCHWEIZER[1]
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Abstract: Protein ubiquitination regulates a variety of cellular events by targeting proteins to proteasomal degradation and/or by modifying the activity, localization and interactions of the modified protein. Neuronal and synaptic physiology depend on the Ubiquitin Proteasome System (UPS). Similarly, alterations of the UPS can be detected in aging, neurological and neurodegenerative diseases. However the role played by protein ubiquitination in synaptic and neuronal function has not been fully elucidated. We have previously reported that in primary neurons both acute pharmacological inhibition of the proteasome and of protein ubiquitination rapidly increase the frequency but not the amplitude of miniature events (minis). These findings lead us to propose that in addition to proteasomal degradation dynamic ubiquitination is critically involved in tuning synaptic activity (Rinetti and Schweizer, 2010). Here, we further investigate this hypothesis. Whole cell patch voltage clamp recording and imaging of vGlut1-pHluorin in primary cortical neurons reveal that acute pharmacological inhibition of protein ubiquitination or protein deubiquitination strongly increases mini frequency but concomitantly reduces action potential triggered responses. These data further suggest that dynamically ubiquitinated proteins orchestrate synaptic activity and indicate that protein ubiquitination can differentially impact on spontaneous and evoked neurotransmission. To uncover the molecular mechanisms underlying such complex regulation we have used a quantitative proteomic approach and identified synaptic proteins whose ubiquitination levels change upon acute UPS inhibition. The results of this study shed light on how protein ubiquitination modulates synaptic function and possibly point to new pathways and targets for the treatment of brain disorders.

Disclosures: **A. Caputo:** None. **K.M. Myers:** None. **A.A. Vashisht:** None. **J.A. Wohlschlegel:** None. **F.E. Schweizer:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

Support: Israel Science Foundation (ISF) Grant no. 1325/08

Title: Compartmentalization of voltage-gated Ca2+ channels in the membrane of rat anterior pituitary cells

Authors: *I. NUSSINOVITCH, E. SOSIAL, A. TZOUR
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Abstract: Voltage-gated Ca^{2+} influx (VGCI) through Ca^{2+} channels plays a key role in the secretion of pituitary hormones. It is well established that L-type Ca^{2+} channels are involved in this VGCI. Whether non-L-type Ca^{2+} channels contribute to this VGCI is unknown. In this study we examined: 1. whether non-L-type Ca^{2+}channels exist in the membrane of pituitary cells. 2. Whether Ca^{2+} channels are segregated among different membrane compartments in the membrane of pituitary cells. Whole-cell recordings and the use of specific Ca^{2+} channel toxin blockers revealed a fraction of non-L-type VGCI that might reach ~46%. Western blotting identified immunblots for; α_{1C}, α_{1D}, α_{1A}, α_{1B} and α_{1E} subunits, corresponding to $Ca_v1.2$, $Ca_v1.3$, $Ca_v2.1$, $Ca_v2.2$, and $Ca_v2.3$ channels. Additionally, RT-PCR identified transcripts for α_{1C}, α_{1D}, α_{1A} and α_{1B} subunits. Transcripts for α_{1E} were non-specific and transcripts for α_{1S} were not detected at all. Taken together these results clearly demonstrate the co-existence of L-type ($Ca_v1.2$ and $Ca_v1.3$) and non L-type ($Ca_v2.1$, $Ca_v2.2$ and $Ca_v2.3$) Ca^{2+} channels in the membrane of anterior pituitary cells. Whether these channels are segregated among different membrane compartments was further investigated in flotation assays using Nycodenz gradients. Western blotting of gradient fractions revealed that $Ca_v1.2$ and $Ca_v1.3$ channels were distributed among light and heavy gradients fractions, i.e., among raft and non raft membrane domains. $Ca_v2.1$ channels were mostly localized in light gradient fractions, i.e., in raft membrane domains whereas $Ca_v2.2$ and $Ca_v2.3$ channels were mostly localized in heavy gradient fractions, i.e., in nonraft membrane domains. In summary, our results demonstrate multiple pathways for VGCI through L-type and non-L-type Ca^{2+} channels in the membrane of native anterior pituitary cells. Compartmentalization of these channels among raft and nonraft membrane domains may underlie their differential regulation by different signalling pathways, under different physiological conditions.

Disclosures: **I. Nussinovitch:** None. **E. Sosial:** None. **A. Tzour:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Program#/Poster#: 402.13/D12

Topic: B.06. Neurotransmitter Release

Support: 1U54NS083924-01 NINDS-NIH

Title: Complexin regulates short-term plasticity of synchronous, asynchronous and delayed quantal release

Authors: ***R. A. JORQUERA**[1,2], E. A. QUIROZ[1]
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Abstract: The timing of neural communication depends on the number of contacts which synchronize the quantal release with the presynaptic action potential. In several chemical synapses the action potential evokes two major forms of release distinguishable by their decay kinetics, the fast synchronous and the slow asynchronous. A different slower form of release known as delayed is induced during high-frequency trains of action potentials decaying after the stimulation cease. Delayed release is thought to be a form of spontaneous release promoted by the resting calcium at the terminals and has been reported as asynchronous release as well. Nevertheless, how all those forms of release are regulated is still under investigation. Complexin binds the SNARE complex fusion machinery, clamps the spontaneous release and promotes the synchronous/asynchronous release. However, the regulatory function of Complexin in the evoked release during the high-frequency nerve-stimulation is not clear. Here we investigate the effects of Complexin in the quantal release kinetics during and after high-frequency nerve stimulation episodes at the *Drosophila* glutamatergic synapses. Complexin null and over-expressions were compared with controls. Quantal release rates were obtained by deconvolution of the evoked-currents recorded by two-electrode voltage-clamp. Our work reveals that the low frequency nerve-stimulation evoked two kinetics of release in Complexin null, control and overexpression, consistent with synchronous and asynchronous release. The synchronous and the asynchronous release at Complexin genotypes display the similar apparent kinetics but different magnitudes, suggesting that the synchronous and the asynchronous release act independently. High-frequency trains of nerve-stimulation evoked three kinetics of release: synchronous, asynchronous and the delayed release. Release rate analysis during the stimulation train indicates

that the synchronous and the asynchronous evoked release display different profile of short-term plasticity. In addition, synchronous and asynchronous short-term plasticity depend on the level of Complexin. The delayed release in Complexin null is earlier activated during the stimulation and decay slower after the stimulation ceases, in turn; Complexin overexpression suppresses this form of release. Our data during high-frequency bursts of nerve activity is consistent with the idea of three form of evoked release with different rates of replenishment and modulated by Complexin.

Disclosures: E.A. Quiroz: None. **R.A. Jorquera:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

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NIH grant EY017836

Title: Complexin 3 regulates vesicle release at a mammalian ribbon synapse

Authors: *L. S. MORTENSEN[1], K. REIM[1], J. KE[2], N. BROSE[1], J. H. SINGER[2], J. RHEE[1]
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Abstract: Complexins (Cplx) are soluble synaptic proteins that exist in four isoforms in mammals. While Cplx1 and 2 are widely expressed in conventional synapses of the brain, the only isoform found at the rod bipolar cell (RBP) to amacrine AII cell ribbon synapse in the retina is Cplx3. All isoforms bind to and stabilize SNARE complexes, and their deletion leads to disturbances in vesicle release. While studies regarding the exact function of Cplx1/2 in conventional synapses have caused some controversy, the function of Cplx3 in mammalian ribbon synapses has not been described. To assess the role of Cplx3 in ribbon synapses, we performed paired recordings from RBP and AII cells in acute retinal slices from Cplx3 genetic deletion mutants. We found that loss of Cpx3 reduced synchronous release while spontaneous release appeared unchanged in Cplx3 KO cells. We simulated a more natural stimulus and determined a reduced release probability, however, multivesicular release is still present despite

the overall desynchronization of vesicle release. Furthermore, short-term depression was reduced at interpulse intervals shorter than 50ms. Taken together, our results suggest that Cplx3 plays an important role in coordinating vesicle release at the retinal bipolar cell - amacrine AII cell synapse.

Disclosures: L.S. Mortensen: None. **K. Reim:** None. **J. Ke:** None. **N. Brose:** None. **J.H. Singer:** None. **J. Rhee:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Program#/Poster#: 402.15/D14

Topic: B.06. Neurotransmitter Release

Title: Spontaneous Ca2+ transients in the nerve terminal of neocortical excitatory neurons

Authors: *V. TRAN[1], C. STRICKER[1,2]
[1]Eccles Inst. of Neurosci., [2]Med. Sch., The Australian Natl. Univ., Canberra, Australia

Abstract: Within the last 15 years, it has become clear that a significant fraction of spontaneous transmitter release can arise from the release of Ca^{2+} from presynaptic stores. However, direct evidence for the role of intracellular Ca^{2+} stores in spontaneous synaptic transmission is still lacking. **Objective:** We hypothesise that, if Ca^{2+} release from stores can trigger transmitter release, then we might be able to detect it as spontaneous increases in cytoplasmic Ca^{2+}. Thus, we image cytoplasmic Ca^{2+} in presynaptic terminals of neocortical pyramidal neurons to investigate whether spontaneous Ca^{2+} transients can be observed. **Methods:** Ca^{2+} imaging is done at 35 ± 1 °C, using a Zeiss LSM 510 laser-scanning confocal microscope. Superficial layer II or layer V pyramidal neurons in the somatosensory cortex of male Wistar rats (P15–19) are filled through the patch pipette with Alexa 568 (50 μM) and Oregon Green 488 BAPTA-1 (OGB-1, 80 μM) for at least 30 min. Synaptic boutons, identified along 1^{st}–3^{rd} order axon collaterals using Alexa 568 fluorescence, are imaged and verified to be release sites based on the presence of a transient increase in OGB-1 fluorescence in response to an evoked AP. OGB-1 fluorescence across the diameter of the bouton is then monitored by line scanning at 10–20 Hz for ~15 min. **Results:** Spontaneous Ca^{2+} transients are observed at a low frequency in both layer II and layer V pyramidal neurons (2 ± 1 and 3 ± 1 mHz, n = 11 and 10 boutons, respectively; p = 0.8). Addition of tetrodotoxin (TTX, 1 μM) to block spontaneous firing does not affect their occurrence (5 ± 2 mHz for layer V pyramids, n = 13; p = 0.3). The average inter-event interval is

12 ± 2 s, with no difference between transients detected in control superfusate and those in TTX (p = 0.3), or between layer II and layer V neurons (p = 0.4). About ½ of all transients occur within 5 s of each other, with some summating to produce a large and extended rise in cytoplasmic Ca^{2+}. The amplitude and decay kinetics of the Ca^{2+} transients are similar between layer II and layer V pyramids ($\Delta F/F = 0.45 \pm 0.05$ *vs.* 0.40 ± 0.05, $\tau_{decay} = 560 \pm 90$ *vs.* 610 ± 100 ms; p = 0.5–0.7), and unaffected by TTX ($\Delta F/F = 0.47 \pm 0.05$, $\tau_{decay} = 680 \pm 70$ ms for layer V neurons; p = 0.4–0.6). However, the amplitude of these spontaneous transients is smaller and their decay time constant longer than those of AP-evoked Ca^{2+} transients ($\Delta F/F = 1.68 \pm 0.05$, $\tau_{decay} = 195 \pm 8$ ms for layer II neurons; $\Delta F/F = 1.19 \pm 0.05$, $\tau_{decay} = 173 \pm 7$ ms for layer V neurons; $p < 10^{-4}$). **Conclusion:** We have detected spontaneously occurring Ca^{2+} transients in nerve terminals of neocortical excitatory neurons. Although to be verified, these transients are likely a result of Ca^{2+} release from presynaptic Ca^{2+} stores.

Disclosures: V. Tran: None. **C. Stricker:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

Support: P50 MH096972

R21 MH77942

Title: Differential transmitter and peptide release modulated by RNA editing of Ca2+-dependent activator protein for secretion (CAPS1)

Authors: *R. ULBRICHT[1], R. LAZARENKO[2], Q. ZHANG[2], R. B. EMESON[3]
[2]Dept. of Pharmacol., [3]Dept. of Pharmacol. and Vanderbilt Brain Inst., [1]Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Ca^{2+}-dependent activator protein for secretion (CAPS1) is required for regulated release from dense core vesicles (DCV) in pheochromocytoma PC-12 cells and promotes DCV exocytosis in chromaffin cells, pancreatic [[Unsupported Character - Symbol Font β]]-cells and neurons. Moreover, the loss of CAPS1 negatively affects synaptic transmission in the mammalian brain. Transcript encoding CAPS1 are subject to adenosine-to-inosine (A-to-I) RNA

editing in which a genomically-encoded adenosine is selectively deaminated to inosine at the level of the pre-messenger RNA. Since inosine base pairs with cytosine, it is recognized as guanosine by cellular machinery to alter a single amino acid within the functionally essential C-terminal domain of CAPS1 from a genomically-encoded glutamine (GAG) to glycine (GIG) codon. Our studies have focused upon defining the role of RNA editing in regulating CAPS1-mediated release. Analyses of RNA from several species showed that RNA editing-mediated changes to CAPS1 coding potential are conserved in vertebrate nervous systems. Quantitative analysis of CAPS1 editing from dissected mouse tissues revealed differential levels of editing ranging from 2% - 25% in peripheral endocrine tissues and selected brain regions. To determine the *cis*- and *trans*-active factors required for A-to-I conversion in CAPS1 transcripts, we used a variety of CAPS1 minigene constructs expressed in heterologous cell culture systems co-expressing one of two mammalian enzymes known to catalyze A-to-I editing (ADARs). Results from our analysis revealed that CAPS1 editing requires intramolecular base pairing of the region surrounding the editing site with a downstream intronic element. This double-stranded RNA duplex is sufficient for editing catalyzed by adenosine deaminase acting on RNA I (ADAR1). Importantly, our work to define the functional implications of CAPS1 editing in primary hippocampal neurons through FM dye loading and unloading as well as mini EPSC recordings suggests that neurotransmission is differentially affected by the expression of edited or non-edited CAPS1 both pre- and post-synaptically. This work provides the first detailed analysis of CAPS1 editing patterns across species and tissues, defines the molecular mechanism underlying this RNA processing event, and indicates its evolutionary significance. Most importantly, these studies provide critical insights into the role that RNA editing plays in the modulation of CAPS1-dependent exocytosis and how dysfunction of this process may contribute to disorders where alterations in hormone/neurotransmitter release have been implicated.

Disclosures: **R. Ulbricht:** None. **R. Lazarenko:** None. **Q. Zhang:** None. **R.B. Emeson:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

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Title: Characterization of voltage-gated Ca2+ channels at individual presynaptic terminals of lamprey reticulospinal axons

Authors: *S. RAMACHANDRAN[1], S. ALFORD[2]
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Abstract: Stimulus-evoked Ca^{2+} influx through voltage-gated Ca^{2+} channels, localized to the presynaptic release face membrane, evokes neurotransmitter release. Differing spatio-temporal Ca^{2+} requirements for release have been proposed in different presynaptic terminals, either gated by Ca^{2+} influx through a large number of open channels or through very few channels, with varying structural relationships to the release machinery. Enumeration of Ca^{2+} channels at a terminal and more importantly the number that open in response to a stimulus is critical to determining the Ca^{2+} requirement for release. Recording Ca^{2+} currents at the release face membrane is thus crucial and recording access to the release face membrane at individual presynaptic terminals an absolute necessity. This has has not been impossible in most presynaptic terminals, including the lamprey reticulospinal synapses. Such recordings have only successfully been achieved in two calyceal presynaptic terminals, which while ideal for recording, represents a rather specialized structure. An acute dissociation of the lamprey spinal cord was developed to obtain viable isolated reticulospinal axons, with functional presynaptic terminals devoid of apposing postsynaptic projections. This presents a significant breakthrough at central synapses, permitting direct recording from the release face membrane of individual terminals. Utilizing the the resultant unrestricted access to the release face membrane afforded by this preparation, Ca^{2+} channels were characterized at individual terminals by immunohistochemistry and low- noise single channel electrophysiological recordings. Multiple subtypes of Ca^{2+} channels, N-, P/Q-, R- and L-type, were found to be present in these terminals. Single channel conductance (10 mM $[Ca^{2+}]_{external}$) was 2.97 ± 0.62 (N), 1.82 ± 0.36 (P/Q), 1.94 ± 0.41 (R), 2.4 ± 0.75 (L) pS. Single channel open probability at 0 mV was 0.26 ± 0.03 (N), 0.29 ± 0.03 (P/Q), 0.22 ± 0.02 (R), 0.24 ± 0.02 (L). Number of channels at a terminal was determined for each of the subtypes; N-type (4-10; mean 6), P/Q-type (3-9; mean 6), R-type (4-32; mean 12) and L-type (3-17; mean 10). A small number of Ca^{2+} channels (up to 68, mean 33) were found to be present at a terminal. Furthermore, a very small number (3-6, mean 4) of Ca^{2+} channels opened in response towith a stimulus, suggesting that Ca^{2+} influx through few open channels may gate release in these terminals. This result, which is the first determination of Ca^{2+} channel activity at single active zone central synapses, has profound implications for the reliability and function of evoked neurotransmitter release in the central nervous system.

Disclosures: **S. Ramachandran:** None. **S. Alford:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Support: R00NS051401

Title: Paired presynaptic and postsynaptic patch clamp recordings to study the effects of action potential kinetics and afterpotentials on synaptic transmission

Authors: *K. G. PARADISO, S. G. CLARKE, A. KISNER
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Abstract: We performed simultaneous presynaptic and postsynaptic recordings to determine how calcium entry produced by various action potential waveforms affects the timing and amplitude of the postsynaptic response in the calyx of Held synapse. Our results indicate that an action potential waveform with physiological kinetics of 0.3 msec depolarization and 0.7 msec repolarization is a close to optimal stimulus to minimize the delay and maximize the amplitude of the postsynaptic response. In separate experiments, we determined the relationship between the action potential repolarization phase and calcium channel response by using voltage jumps to +60 mV at various times during and after the repolarization. This indicated that calcium influx continues for a short duration after the action potential has returned to baseline. We therefore tested if afterpotentials that immediately follow a stimulus can affect calcium channel activity. We tested a box stimulus having an instant repolarization and compared that to an action potential-like waveform having a physiologically relevant 0.7 msec ramped repolarization. For both stimuli, the repolarization ended at baseline or ended in a -20 mV or +20 mV afterpotential. As expected, based on electrical driving force, instant current jumps show a difference in calcium channel response to both hyperpolarizing and depolarizing afterpotentials. Compared to traces without an afterpotential, a 20 mV afterhyperpolarization acts to slightly increase the peak calcium current and slightly shorten the decay phase of the calcium channel response, while a 20 mV depolarizing afterpotential does the opposite. Paired pre- and postsynaptic recordings with instant jumps demonstrated that depolarizing afterpotentials produced a larger postsynaptic response than hyperpolarizing afterpotentials. Consistent with this, the integral of the calcium channel response to an instant repolarization is larger in the presence of an afterdepolarization compared to the response in the presence of an afterhyperpolarization. In contrast, the ramped 0.7 msec repolarization did not appear to show a difference in calcium channel activity in the presence or absence of an afterpotential. In addition, paired recordings using a 0.7 msec ramp repolarization do not appear to show a statistically significant difference in the postsynaptic response when the presynaptic waveform ends in a hyperpolarized or depolarized afterpotential. This indicates that the physiological repolarization rate acts to prolong the response, and this acts

to reduce or prevent afterpotentials from affecting the calcium channel response and subsequent synaptic transmission.

Disclosures: K.G. Paradiso: None. **S.G. Clarke:** None. **A. Kisner:** None.

Poster

402. Neurotransmitter Release: Docking, Fusion, and Calcium Dependence

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Topic: B.06. Neurotransmitter Release

Support: American Heart Association 11BGIA7430033

NIDCD RO1, DC013157

Title: Reverse operation of NCX mediates presynaptic Ca2+ transient and spontaneous glutamate release during *in vitro* ischemia

Authors: *S. LEE, J. KIM
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Abstract: An early cellular consequence of brain ischemia is an increase in vesicular glutamate release from presynaptic terminals in the central nervous system (CNS). However, the underlying mechanisms of increased vesicular glutamate release at presynaptic terminals during the early stage of ischemia are still controversial. Here we studied presynaptic responses and mechanisms of increased glutamate release during ischemia, using pre- and postsynaptic whole-cell recordings and presynaptic Ca2+ imaging at the calyx of Held synapse in rat brainstem slices. *In vitro* ischemia, induced by oxygen glucose deprivation and addition of 2 mM iodoacetic acid (IAA, an inhibitor of glycolysis), significantly increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) without changing in the amplitude of sEPSCs, indicating that ischemia enhances vesicular glutamate release from the calyx of Held terminal. We found that the ischemia-induced vesicular glutamate release was dependent on an increase in presynaptic intracellular Ca2+ ([Ca2+]i), resulting from extracellular Ca2+ influx. During ischemia, increased Ca2+ influx to the presynaptic terminal is associated with the reverse operation of Na+/ Ca2+ exchanger (NCX) rather than presynaptic depolarization or voltage-activated Ca2+ currents. KB-R7943, an inhibitor of NCX, prevented the increase of presynaptic [Ca2+]i and vesicular glutamate release during *in vitro* ischemia. In addition, presynaptic Na+

accumulation using monensine (50 μM) facilitated reverse operation of NCX and consequently increased the frequency of sEPSCs that were inhibited by KB-R7943. These results suggest that the disruption of Na+ and Ca2+ dynamics causes a rise in presynaptic [Ca2+]i and an increase in vesicular glutamate release during the early phase of brain ischemia.

Disclosures: S. Lee: None. **J. Kim:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

Location: Halls A-C

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Topic: B.06. Neurotransmitter Release

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Start up fund from GRU

Title: Requirement of membrane cholesterol in vesicle endocytosis at a mammalian central synapse

Authors: H.-Y. YUE, *J. XU
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Abstract: Membrane cholesterol has been indicated important in constitutive endocytosis and receptor-mediated clathrin-dependent endocytosis of non-neuronal cells, but its role in synaptic vesicle endocytosis remains unclear. We investigated this issue at the rat calyx of Held synapse by monitoring endocytosis with the whole-cell membrane capacitance measurement. Depleting cholesterol with methyl-β-cyclodextrin (MβCD) dialysis via the patch-clamp pipette led to significant inhibition of both rapid and slow endocytosis. Bath application of MβCD similarly inhibited endocytosis. Such inhibition of endocytosis could take place without any changes in the voltage-gated calcium channel current or exocytosis. Together, these results provide direct evidence that membrane cholesterol regulates vesicle endocytosis at the mammalian central synapse, probably downstream of Ca2+ entry and vesicle exocytosis.

Disclosures: H. Yue: None. **J. Xu:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Topic: B.06. Neurotransmitter Release

Support: MRC grant G1002117

BBSRC studentship

Title: Synaptic vesicle phluorins differentially report distinct endocytic routes

Authors: *J. C. NICHOLSON-FISH[1], K. J. SMILLIE[1], M. A. COUSIN[2]
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Abstract: Upon depolarisation of a presynaptic terminal synaptic vesicles (SVs) fuse with the neuronal plasma membrane, thereby releasing their contents into the synaptic cleft in a process called exocytosis. Endocytosis is tightly coupled to exocytosis to ensure retrieval of SV membrane and to recycle deposited SV proteins. Endocytosis has two main modes: clathrin-mediated endocytosis (CME), which forms single SVs via the action of AP-2 and a clathrin coat and activity-dependent bulk endocytosis (ADBE), which invaginates a larger area of plasma membrane to form an endosome which later generates SVs. The kinetics of CME can be monitored by expressing exogenous SV proteins fused to pH-sensitive variants of green fluorescent protein (pHluorins). This enables the visualisation of the traffic of specific SV cargo as a proxy of the rate and extent of exo- and endocytosis. These pHluorin reporters are routinely employed to monitor CME, however little is known regarding how (and if) they report ADBE. We appraised the ability of widely used pHluorin reporters to monitor ADBE in primary neuronal cultures. To discriminate between CME and ADBE we employed a series of pharmacological inhibitors and genetic manipulations which arrested each endocytosis mode. We revealed that inhibition of ADBE has little effect on the response of a range of widely used pHluorin reporters, whereas manipulation of CME produced dramatic effects. Intriguingly we have identified one SV cargo that does report ADBE when fused to pHluorin, but does not report CME. Thus we have revealed preferential trafficking of specific SV proteins through different endocytic modes, and in turn we have identified a novel genetic reporter of ADBE.

Disclosures: J.C. Nicholson-Fish: None. **K.J. Smillie:** None. **M.A. Cousin:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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the Center for Nanoscale Microscopy and Molecular Physiology of the Brain (T.M. and S.O.R)

Title: Disruption of adaptor protein 2μ in hair cells impairs vesicle replenishment and hearing

Authors: S. JUNG[1,2], T. MARITZEN[3,4], C. WICHMANN[2,5], Z. JING[2,6], N. H. REVELO[2,7], H. EL-MOYED[2,5], S. MEESE[2,8], S. M. WOJCIK[9], I. PANOU[1,2], P. SCHU[10], R. FICNER[2,8], E. REISINGER[2,11], S. RIZZOLI[2,7,12], J. NEEF[1,2], N. STRENZKE[2,6], V. HAUCKE[3,4], *T. MOSER[1,2,12]

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Abstract: The presynaptic active zone (AZ) of cochlear inner hair cells (IHCs) sustains vesicle release at hundreds per second. This requires the function of otoferlin, a C2 domain protein defective in human deafness. Here we show that the endocytic adapter protein 2 (AP-2) directly associates with otoferlin and is required for vesicle replenishment and hearing. We demonstrate that conditional deletion of the Ap-2μ gene in mouse IHCs causes a use-dependent synaptic hearing impairment. Membrane capacitance measurements in IHCs and extracellular recordings from single spiral ganglion neurons (SGNs)show that AP-2μ-deficient IHCs are unable to sustain exocytosis already at 20 ms of stimulation. This phenotype reflects defective replenishment of the readily releasable pool of vesicles (RRP) despite unaltered membrane retrieval and a normal number of membrane-proximal vesicles at the AZ. It qualitatively resembles that of pachanga otoferlin mutants. This similarity and the fast onset lead us to suggest that AP-2 binding to otoferlin helps to clear newly exocytosed material from the release sites for the new coming vesicles to become fusion-competent (AZ clearance). Moreover, electron and super-resolution microscopy revealed an accumulation of endosome-like vacuoles (ELVs), impaired vesicle regeneration from ELVs and a concomitant depletion of ribbon-associated synaptic vesicles during long lasting stimulation of AP-2μ–deficient IHCs. We conclude that indefatigable sound coding at IHC ribbon synapses depends on clearance of newly exocytosed material from release sites to the perisynaptic endocytic zone via AP-2/ otoferlin and AP-2 dependent vesicle regeneration from ELVs.

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Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Topic: B.06. Neurotransmitter Release

Support: ANPCyT PICT 2011 N° 2667, Argentina

Title: pH regulation by carbonic anhydrase modulates synaptic vesicle release mode at the mouse levator auris longus neuromuscular junction

Authors: *O. D. UCHITEL, A. I. GROISMAN
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Abstract: Acetazolamide (AZ) is known to inhibit the action of carbonic anhydrase (CA), an enzyme responsible for regulating the extra- and intracellular pH. AZ is used to treat certain types of epilepsy and ataxia but its mechanism of action is still unknown. Our objective is to better understand the AZ mechanism of action on synaptic transmission vesicle recycling. Electrophysiological recordings performed on *ex vivo* levator auris longus neuromuscular junctions (NMJs) treated with AZ (100 uM in bicarbonate solution) showed reduced amplitude and increased frequency of spontaneous end plate potentials. Evoked end plate potential amplitude was also reduced but quantal content (m) measured at low, high frequency and during burst stimulation showed no differences between treated and control NMJs. To get further insight into the role of CA in transmitter release the above experiments were repeated in a Hepes buffer solution (10mM, pH 7.4). In those conditions m was not affected by AZ. The dynamics of vesicle exocytosis/endocytosis were studied in bicarbonate buffer solution loading the synaptic vesicle with fluorescence FM 2-10 dye incubated before and after 10 min nerve stimulation (20Hz) and unloading with a 50 Hz continuous stimulation after dye washed. The amount of dye loaded in the presence of AZ was reduced to 48±9% of control. After loading the nerve terminal in control conditions, muscles were treated with AZ and unloaded in the presence of the drug. Different kinetics of dye release were observed with a fluorescence retention of 68±6% vs. 24±4% in treated vs. control NMJs. Unloading experiments were repeated in a Hepes buffer (10mM, pH 7.4) and no effect of AZ was observed (26±4% at treated NMJs vs 23±2% of control). These results suggest that alterations in the buffer capacity of the nerve terminal by inhibition of CA affect vesicle recycling. Furthermore, the fact that in similar conditions AZ reduces dye release without affecting m suggests that intracellular pH changes may affect the mode of synaptic vesicle exocytosis.

Disclosures: O.D. Uchitel: None. **A.I. Groisman:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Topic: B.06. Neurotransmitter Release

Support: NS00085

Startup fund from GRU

Title: Regulation of vesicle cycling by myosin light chain kinase at hippocampal boutons

Authors: L. LI, H.-Y. YUE, *Y.-C. ZHU, J. XU
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Abstract: Synaptic transmission during repetitive activity is maintained by vesicle cycling via endocytosis at nerve terminals. Vesicle cycling depends on Ca2+/calmodulin, and, their downstream myosin light chain kinase (MLCK) according to our recent study at the calyx of Held synapse. Whether MLCK regulates endocytosis and vesicle cycling in small conventional synapses remains unclear. To address this question, we study effects of MLCK inhibition on vesicle exocytosis and endocytosis at cultured hippocampal boutons by imaging the dynamics of pHluorin-tagged synaptobrevin or synaptophysin. MLCK inhibitors, ML-7 and wortmannin, slowed the kinetics of endocytosis following electrical stimulation. Because MLCK inhibitors may have non-specific inhibition on calcium channel current, we are now studying effects of reducing MLCK expression by RNA interference-mediated gene silencing. Our results will help to further elucidate the presynaptic functions of MLCK in neurotransmission.

Disclosures: L. Li: None. **H. Yue:** None. **Y. Zhu:** None. **J. Xu:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Topic: B.06. Neurotransmitter Release

Support: RO1 MH099557

U54 NS083925

Title: Synaptic vesicle recycling is enhanced in complexin deficient *Drosophila* neuromuscular junctions

Authors: *N. SABEVA, A. GONZALEZ, M. BYKHOVSKAIA
Univercidad Central Del Caribe, Bayamon, Puerto Rico

Abstract: Complexin (Cpx) is a small cytosolic protein that interacts with the SNARE complex and regulates the final stages of exocytosis. Cpx null mutation in *Drosophila* produces drastic enhancement in spontaneous synaptic activity, suggesting that Cpx prevents spontaneous exocytosis. To understand how synaptic vesicle recycling can compensate for this increased activity in Cpx null mutants, we combined activity dependent loading of the fluorescent marker FM1-43 with electron microscopy analysis. First, we tested whether evoked and spontaneous release components in cpx(-) neuromuscular junctions (NMJs) are associated with different recycling pools of vesicles. The NMJs were loaded with the dye either in the absence of stimulation (passive staining) or during electrical stimulation at a 5 Hz frequency (active staining) and unloaded in the absence of stimulation (passive destaining). We found that the recycling pool of vesicles was significantly increased in cpx(-) NMJs and that the fluorescence loss during passive destaining was similar at the terminals loaded actively or passively. This result suggests the recycling pool is increased in cpx(-) terminals to compensate for the enhanced spontaneous release and that the recycling pools of vesicles participating in the evoked and spontaneous release components are intermixed. To test this hypothesis further, we employed photoconversion of FM1-43 loaded passively at different times ranging from 10 s to 10 min. We found that the number of FM1-43 loaded synaptic vesicles in cpx(-) NMJs was significantly increased at every time point and that the recycling and resting vesicles were spatially intermixed. These results suggest that the vesicle pools involved in evoked and spontaneous recycling pathways are mixed in the *Drosophila* NMJs and that cpx(-) synaptic boutons have an increased recycling pool to enable elevated spontaneous fusion.

Disclosures: **N. Sabeva:** None. **M. Bykhovskaia:** None. **A. Gonzalez:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Dystonia Medical Research Foundation

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Whitehall Foundation

Title: Enhanced synaptic vesicle recycling in cultured striatal neurons of DYT1 dystonia mouse model

Authors: *N. C. HARATA, S. IWABUCHI, J.-Y. KOH
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Abstract: Dystonia is a hyperkinetic movement disorder and is characterized by sustained or intermittent muscle contractions that cause abnormal and often repetitive movements, postures, or both. Patients with dystonia are subject to abnormal neuronal function in the brain, in the absence of neurodegeneration. Thus, one of the main pathophysiological causes is thought to be abnormal synaptic transmission, e.g. in the striatum, a region of the mammalian brain that plays an important role in motor control. However, whether this abnormality arises pre- or postsynaptically remains unclear. Here we have tested the striatal neurons of a mouse model of dystonia for abnormalities in presynaptic signaling. Specifically, we prepared primary cultures of striatal neurons from a knock-in model of DYT1 dystonia, the most common form of inherited dystonia. We evaluated the recycling of synaptic vesicles in the nerve terminals by live-cell imaging of the recycling marker FM1-43. Compared to wild-type controls, the mutant neurons showed an enhancement of both the amount of dye that was loaded in response to a stimulus, and the rate at which the dye was released from the loaded nerve terminals. Moreover, the amount of dye released from the mutant nerve terminals was higher even during miniature synaptic activity when action potentials were blocked with the Na+ channel blocker tetrodotoxin. Our results demonstrate that synaptic vesicle recycling is enhanced during both evoked and miniature synaptic activity in the mutant striatal neurons. In addition, they indicate that, at a minimum, a presynaptic factor is affected in DYT1 dystonia.

Disclosures: **N.C. Harata:** None. **S. Iwabuchi:** None. **J. Koh:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Title: A novel membrane-binding probe for the morphological and molecular characterization of synaptic vesicle recycling pathways

Authors: *N. H. REVELO, S. TRUCKENBRODT, S. O. RIZZOLI
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Abstract: The molecular composition of organelles involved in membrane recycling has been difficult to study due to the lack of suitable labeling tools. Electron microscopy approaches, such as those combining immunogold labeling and endocytosis tracers, have several drawbacks, including laborious sample preparation, low immunolabeling density, and low throughput. Fluorescence microscopy studies have tracked endocytosis by using amphiphatic dyes, rendered fixable by the addition of one amine group. We have found that these probes largely detach from membranes after permeabilization procedures, thus not allowing the use of immunofluorescence labeling to characterize endocytotic organelles. To circumvent these difficulties we have developed a novel probe, named mCLING (membrane-binding fluorophore-Cysteine-Lysine-Palmitoyl Group), which contains seven amine groups for better aldehyde fixation, a lipidic tail that binds to the plasma membrane, and a fluorophore suitable for high-resolution microscopy. mCLING is not toxic and is efficiently taken up during endocytosis. Moreover, mCLING remains attached to membranes after fixation and permeabilization, and can therefore be used in combination with immunolabeling. mCLING was tested in mammalian cultured cells, yeast and the *Drosophila* larval neuromuscular junction, finding efficient membrane labeling and uptake. We then applied mCLING to cultured hippocampal neurons in order to answer open questions about synaptic vesicle recycling and protein organization: 1) are the same synaptic vesicles responsible for active and spontaneous release? We found that actively and spontaneously-released synaptic vesicles are different in protein composition, the latter being more related to constitutive endosomal traffic. 2) What is the fraction of synaptic vesicle proteins that remains stranded on the plasma membrane, possibly as a readily retrievable pool of vesicles? Surface labeling with mCLING combined with immunolabeling of endogenous synaptic proteins allowed us to establish that ~12 to 22% of them remain on the plasma membrane. 3) What is the organization of SNAP-25 and syntaxin 1 on intracellular organelles? So far clusters of these proteins have only been studied on the plasma membrane. Using mCLING as a surface marker, it was possible to establish that SNAP-25 forms clusters of similar size on the plasma membrane and in intracellular organelles. In contrast, Syntaxin 1 forms larger clusters on the plasma membrane. We conclude that mCLING enables the investigation of trafficking membranes in a broad range of preparations.

Disclosures: **N.H. Revelo:** None. **S. Truckenbrodt:** None. **S.O. Rizzoli:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Support: NIH NINDS NS029051

Title: Hsp70 generates force to dissociate clathrin coats by an osmotic-pressure mechanism that is amplified by Hsp70 self-association

Authors: ***E. M. LAFER**, S. JIN, R. SOUSA
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Abstract: Hsp70 molecular chaperones carry out a wide array of protein processing reactions in cells including protein translocation into the ER and mitochondrion, disaggregation of heterogeneous aggregates and disassociation of protein complexes. The mechanism by which Hsp70s generate force to execute these mechanical processes remains unclear, but is especially amenable to study in a reaction that is central to synaptic function: the Hsc70-mediated uncoating of the clathrin coated vesicles formed upon endocytosis at nerve terminals. Unlike heterogeneous aggregates, clathrin coats are homogeneous particles with defined Hsc70-binding sites presented with unique geometry inside the coat structure. We have taken advantage of these features to alter the geometry of these binding sites and to swap them with binding sites for other proteins so as to allow explicit testing of different models for Hsp70 force generation in protein complex dissociation. Our results allow us to reject models in which the Hsp70 needs to undergo a conformational 'power stroke' to generate force, and they also argue against simple 'thermal fluctuation capture' (Brownian ratchet) models. Instead, our results indicate that forces of up to tens to hundreds of piconewtons are generated by a mechanism analogous to that which generates osmotic pressure, and which provides for momentum exchange between the clathrin structural wall and Hsp70s tethered to flexible polypeptide segments adjacent to this wall. We also find that this force is amplified by Hsp70 self-association to allow dissociation of exceptionally stable clathrin coats, suggesting a biological function for the self-associating properties of these ubiquitous chaperones. In the broadest perspective, our data address how information processing in the brain is vastly more energy efficient than in human engineered

information processing machines by highlighting how biological machines, rather than expending energy in suppressing noise and fluctuations, exploit them.

Disclosures: **E.M. Lafer:** None. **S. Jin:** None. **R. Sousa:** None.

Poster

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Support: NIMH R01 MH083691

IMHRO

NARSAD

Title: Trafficking of the vesicular glutamate transporter, VGLUT2

Authors: ***H. LI**, M. SANTOS, K. PARK, S. M. VOGLMAIER
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Abstract: The two principal vesicular glutamate transporter isoforms, VGLUT1 and 2, are essential for filling synaptic vesicles with the major excitatory neurotransmitter glutamate and have been suggested to confer distinct physiological properties of glutamate release in synapses where these isoforms are complementarily expressed. We investigated isoform-specific recycling to understand how differences in recycling of the two VGLUT isoforms might account for differences in exocytosis. We initially observed that VGLUT2 responds differently to high frequency stimulation than VGLUT1. To further explore trafficking differences of VGLUT2 from its closely related VGLUT1 isoform, we utilize a pHluorin-based reporter, VGLUT2-pH. VGLUT2-pH exhibits different recycling dynamics than VGLUT1-pH, even in response to moderate stimulation, reflected by slower rates of exocytosis and endocytosis. We explore how these differences are related to synaptic vesicle pools with differing probabilities of release. In addition, we characterize the crucial role of a C-terminal dileucine-like motif for VGLUT2 trafficking. Disruption of this motif abolishes the synaptic targeting of VGLUT2 and essentially eliminates the endocytosis of the transporter protein. Mutational and biochemical analysis demonstrates that clathrin adaptor proteins interact at the C-terminal dileucine-like motif of VGLUT2. This motif is crucial for the interaction of VGLUT2 with AP-2, a well-studied adaptor

protein required for clathrin mediated endocytosis. However, VGLUT2 also specifically interacts with AP-1 and -3 at the dileucine-like sorting signal. VGLUT2-pH relies on distinct recycling mechanisms from VGLUT1 under conditions of high frequency or sustained stimulation. Abrogation of these differences by pharmacological and molecular inhibition reveals that these mechanisms are dependent on the adaptor proteins AP-1 and AP-3. Further, shRNA-mediated knockdown demonstrates individual roles for AP-1 and AP-3 in VGLUT2 recycling.

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Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Spain MICINN BFU2009-12160

Title: Parallel operation of slow and fast releasing readily releasable pools at calyx of Held synapses

Authors: K. MAHFOOZ[1], R. RENDEN[2], *J. F. WESSELING[1]
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Abstract: The readily releasable pool (RRP) of vesicles is a key concept in many studies of presynaptic function. The motivating idea is that readily releasable vesicles can participate in neurotransmission on demand. However, fast and slowly releasing subdivisions within the RRP have been identified, and it has been suggested that slow vesicles may be biochemically upstream of the immediately releasable state owing to incomplete priming. Nevertheless, here we use abrupt jumps in presynaptic action potential firing frequency from 100 to 300 Hz to show that slow vesicles are immediately available for synchronous release at intact calyces of Held from 2-3 week old mice and rats, albeit at lower probability of release compared to fast vesicles. In addition, other operating principles were more similar to standard excitatory synapses than expected: when slow vesicles were included, the RRP had a fixed capacity that did not depend on extracellular Ca2+ levels; and activity-dependent acceleration of recruitment of new vesicles reached a maximum quickly. Key differences were confirmed, including order-of-magnitude

more acceleration at calyces and the absence of a powerful short-term enhancement mechanism termed augmentation. Overall, the results support the simplifying concept that the RRP consists of a collection of independent release sites operating in parallel, with a range of release probabilities.

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Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

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Topic: B.06. Neurotransmitter Release

Support: NSC

Title: SNAP-25 phosphorylation modulates the exocytotic kinetics in secretory cells and the large-scale network activity in the developing rat retina

Authors: *Y.-T. HSIAO, C.-C. YANG, Y.-T. HUANG, C.-T. WANG
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Abstract: SNARE complex mediates vesicle fusion. Among three SNARE proteins, SN25 can be phosphorylated by protein kinase A (PKA) at the residue of T138. Previous studies showed that SN25 phosphorylation by PKA can regulate secretion via recruiting vesicles. However, it remains unclear (1) how SN25 phosphorylation regulates the kinetics of exocytosis and (2) whether this regulation can cause a significant effect on the large-scale network activity. To address the first question, we performed single-event amperometry in PC12 cells to study the effects of SN25 phosphorylation on the exocytotic kinetics, with a special focus on the dynamics of fusion pore, reflected by foot signals preceding amperometry spikes (prespike foot, PSF). Two different secretogogues were applied to trigger calcium-dependent exocytosis, including KCl alone and KCl with forskolin. KCl alone did not change the secretion rate in cells overexpressing SN25 compared to control, but KCl with forskolin reduced the secretion rate in cells overexpressing SN25 compared to control, suggesting that SN25 may down-regulate calcium-dependent exocytosis via PKA phosphorylation. Furthermore, the secretion rate was increased in cells overexpressing the SN25 phosphodeficient mutant (SN25-T138A, designated SN25-PKA*) compared to SN25, confirming that SN25 down-regulates the secretion rate via the PKA phosphorylation site. Moreover, KCl with forskolin reduced PSF lifetime in cells overexpressing

SN25-PKA* compared to SN25, suggesting that SN25 phosphorylation stabilizes fusion pore. Thus, SN25 phosphorylation may regulate the kinetics of exocytosis in secretory cells. To address whether SN25 phosphorylation can cause a significant effect on the large-scale network activity, the spontaneous, correlated patterned activity (termed retinal waves) was detected in the developing rat retina where the PKA activity remains high. Live calcium imaging was subsequently performed to monitor the wave-associated calcium transients after overexpressing SN25 or SN25-PKA* in presynaptic neurons. The frequency of retinal waves was reduced by overexpressing SN25 in presynaptic neurons, whereas SN25-PKA* did not change the wave frequency, suggesting that SN25 phosphorylation by PKA may down-regulate the wave activity. Together, our results suggest that post-translation modification at a single residue of SN25 may serve as a molecular regulator to modulate neurotransmitter release and the large-scale network activity.

Disclosures: **Y. Hsiao:** None. **C. Yang:** None. **Y. Huang:** None. **C. Wang:** None.

Poster

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Alzheimer's Association (Reed - NIRG-12-242187)

Title: Increased glutamate release in the CA3 correlates with memory deficits in mice expressing P301L tau

Authors: ***H. C. HUNSBERGER**, C. C. RUDY, D. S. WEITZNER, M. N. REED*
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Abstract: Individuals at risk for developing Alzheimer’s disease (AD) often exhibit neuronal network hyperexcitability, particularly in the CA3 region of the hippocampus, in the years preceding AD diagnosis. Recently, the microtubule-binding protein tau has been implicated in this hyperexcitability. The exact mechanism by which tau produces hyperexcitability remains to be determined, but recent work suggests tau may mediate glutamatergic signaling. We show that rTg(TauP301L)4510 mice, a commonly used tau mouse model of AD, exhibit a 65% increase in

hippocampal vesicular glutamate transporter (VGLUT) expression. Because VGLUTs impact the amount of glutamate released into the synapse and increased VGLUT expression causes excitotoxic neuro-degeneration, we hypothesized that the neurodegeneration observed in our AD model is due in part to an increase in extracellular glutamate levels. To examine glutamate regulation *in vivo* in TauP301L mice, we used an amperometry coupled to ceramic-based microelectrode arrays (MEAs), which allows for measurement of glutamate levels. Glutamate regulation was measured separately in the dentate gyrus (DG), CA3 and CA1 regions of the hippocampus and correlated with memory performance in the hippocampus-dependent Barnes maze. To control for the overexpression of human tau, we also examined glutamate regulation in mice expressing wild-type human tau (TauWT) at levels equivalent to that of mice expressing P301L tau (TauP301L). TauP301L mice exhibited memory deficits in the Barnes maze. P301L tau expression did not affect tonic extracellular glutamate levels or clearance from the synapse in any region examined. Similarly, amplitudes of potassium-evoked glutamate release in the DG and CA1 were similar among TauP301L, TauWT, and transgene negative littermates. However, memory-impaired TauP301L mice exhibited a 7-fold increase in glutamate release in the CA3 region of the hippocampus, and spatial reference memory errors correlated with potassium-evoked glutamate release in the CA3. In addition, the glutamate clearance peak area, area under the curve, was significantly greater for TauP301L mice in the DG and CA3 regions of the hippocampus. These data suggest a possible novel mechanism, increased presynaptic glutamate release, by which tau may mediate hyperexcitability. Findings of increased CA3 glutamate release in our mouse model corroborate findings of CA3 hyperexcitability in memory-impaired aged humans and patients with mild cognitive impairment (MCI). We are currently examining whether decreasing glutamate release can attenuate memory deficits and AD-related pathology in the TauP301L mouse model of AD.

Disclosures: H.C. Hunsberger: None. **C.C. Rudy:** None. **D.S. Weitzner:** None. **M.N. Reed*:** None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 403.14/D32

Topic: B.06. Neurotransmitter Release

Support: NIH Grant 1SC1NS077776

NIH Grant 5G12RR003035-27

NIH Grant 8G12MD007583-27

Title: Readily releasable vesicles recycle at the active zone of hippocampal synapses

Authors: *T. A. SCHIKORSKI
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Abstract: During the synaptic vesicle cycle, synaptic vesicles fuse with the plasma membrane and recycle for repeated exo/endocytic events. By using activity-dependent FM1-43 dye uptake combined with fast (<1s) microwave-assisted fixation followed by photoconversion and ultrastructural 3D-analysis, we tracked endocytic vesicles over time, “frame-by-frame”. The first retrieved synaptic vesicles appear 4s after stimulation and these endocytic vesicles are located just above the active zone. Second, the retrieved vesicles did not show any sign of a protein coat and coated pits were not detected. Between 10s and 30s, large labeled vesicles appeared that had up to 5 times the size of an individual synaptic vesicle. Starting at around 20s these large labeled vesicles decreased in number in favor of labeled synaptic vesicles and after 30s labeled vesicles re-dock at the active zone. The data suggest that readily releasable vesicles are retrieved as non-coated vesicles at the active zone.

Disclosures: T.A. Schikorski: None.

Poster

403. Neurotransmitter Release: Vesicle Recycling and Biogenesis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 403.15/D33

Topic: B.06. Neurotransmitter Release

Support: R01 MH 084874

Title: Monte Carlo simulation of transmitter release and receptor activation at CA1 glutamatergic synapses supports modified fusion dynamics as a mechanism of 5-HT1BR inhibition

Authors: *E. C. CHURCH[1], E. HAMID[1,3], S. ALFORD[2]
[1]Grad. Program in Neurosci., [2]Biol. Sci., Univ. of Illinois At Chicago, Chicago, IL; [3]NIH, Washington, DC

Abstract: Both 5-HT_{1B} receptors and $GABA_B$ receptors cause presynaptic inhibition and are colocalized at CA1-subicular pyramidal cell synapses. This $G_{i/o}$-mediated presynaptic inhibition is widely believed to require a reduction of release probability (P_r) following inhibition of evoked Ca^{2+} entry. However, GPCRs may inhibit neurotransmission independently of Ca^{2+} channel effects. Indeed, whereas presynaptic $GABA_B$ receptors inhibit presynaptic Ca^{2+} entry, 5-HT_{1B} receptors similarly inhibit neurotransmission from CA1 neurons but this action is mediated by a direct targeting of G[[Unsupported Character - Symbol Font ]][[Unsupported Character - Symbol Font ]] to the presynaptic release machinery (Hamid et al. 2014). We demonstrate that $GABA_B$ receptors alter P_r, whereas 5-HT_{1B} receptor activation leaves P_r unaltered, but reduces peak evoked cleft glutamate concentration. This modulation of cleft glutamate concentration allows a dynamic modulation of postsynaptic glutamate receptors, such that 5-HT_{1B} receptors are much less effective at inhibiting NMDA receptor-mediated synaptic responses than those mediated by AMPA receptors. Changes in peak glutamate cleft concentrations cannot be caused by a reduction of P_r spread over a number of synapses that exhibit univesicular release, but may be caused either by a reduction of P_r of multivesicular release sites or by a restriction of glutamate release from a fusion pore. A three dimensional simplified model of a synaptic cleft was thus created to run simulations in mcell (Stiles et al. 1998) of the effect of release of glutamate from a single vesicle in which fusion was arrested at pore sizes ranging from 0.4 nm to full fusion, or in which release was modelled from between 1 to 3 vesicles into a single synaptic cleft (modeled as a 300 nm disc). At 300 nm synaptic clefts, a restricted fusion pore model was substantially more accurate and reliable in predicting inhibition of AMPA currents modeled on activation of a 4 ligand kinetic model (Robert and Howe 2003) and a failure to inhibit a 2 ligand binding site of the NMDA receptor (Banke and Traynelis 2003), suggesting a likely mechanism of action for 5-HT_{1B}R-mediated synapic inhibition.

Disclosures: E.C. Church: None. **E. Hamid:** None. **S. Alford:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Program#/Poster#: 404.01/D34

Topic: B.08. Synaptic Plasticity

Support: NIH Grant ES020465

Title: Early developmental exposure to lead chronically inhibits vesicular release and alters presynaptic ultrastructure at excitatory synapses in the adult rat hippocampus

Authors: *P. K. STANTON[1], X.-L. ZHANG[1], S. R. GUARIGLIA[2], K. H. STANSFIELD[2], J. L. MCGLOTHAN[2], T. R. GUILARTE[2]
[1]New York Med. Col., Valhalla, NY; [2]Columbia Univ., New York, NY

Abstract: Lead exposure during brain development inhibits neurotransmitter release and this effect is likely to contribute to impaired synapse formation, plasticity and learning deficits. However, the mechanism(s) by which lead impairs neurotransmitter release have not been fully elucidated. In primary hippocampal neurons *in vitro*, lead exposure inhibits vesicular release and reduces the number of fast-releasing sites, an effect mediated by NMDAR inhibition. We wanted to determine if similar effects were also present in animals exposed to lead *in vivo*. In the present study, we examined the effects of chronic lead exposure on presynaptic transmitter release using two-photon laser scanning microscopy of FM1-43 vesicular release in Schaffer collateral-CA1 synapses in *in vitro* hippocampal slices. We found that chronic lead exposure significantly enhanced paired-pulse facilitation (Control PPF = 1.7 versus Lead PPF = 3.0 at 30msec inter-pulse interval), an indirect measure of reduced release probability. Mean-variance analysis of evoked synaptic potentials at Schaffer collateral-CA1 synapses directly confirmed that chronic lead treatment persistently reduced transmitter release probability (Control Pr = 0.46; Pb Pr = 0.25 at 2mM [Ca2+]o). Using FM1-43 2-photon imaging of release from CA1 Schaffer collateral terminals, we found that this reduced probability was associated with reduced release of glutamate from vesicles in the rapidly-recycling vesicle pool loaded by hypertonic shock. Furthermore, using transmission electron microscopy, we found that Schaffer collateral-CA1 terminals had fewer vesicles in the rapidly-recycling vesicle pool, decreased docked vesicle density at the presynaptic active zone and a reduced number of presynaptic terminals with multiple mitochondria. Similarly, in mossy fiber-CA3 synapses, we observed fewer vesicles in the rapidly-recycling pool, a decreased docked vesicle density and a shortened postsynaptic density length. These studies extend our previous finding in primary hippocampal neurons that lead produces profound impairments in vesicular release that are likely to contribute to deficits in synaptic plasticity necessary for cognitive function.

Disclosures: P.K. Stanton: None. **X. Zhang:** None. **S.R. Guariglia:** None. **K.H. Stansfield:** None. **J.L. McGlothan:** None. **T.R. Guilarte:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.02/D35

Topic: B.08. Synaptic Plasticity

Support: R01NS080851 (PSH)

R01NS046072 (RJT)

Title: Neurophysiological responses of recovery in pediatric mice compared to adult mice with transient focal cerebral ischemia

Authors: *J. E. ORFILA, H. GREWAL, T. SHIMIZU, R. T. TRAYSTMAN, P. S. HERSON
Univ. of Colorado, Anschutz Med. Campus, Aurora, CO

Abstract: Ischemic stroke is the fourth leading cause of death in the United States and is increasingly being recognized as a disease that strikes people of all ages, not just the elderly. Studies suggest that the immature developing brain may have a greater degree of plasticity compared to the adult, thereby enhancing functional recovery to a greater extent during development. However, this logical hypothesis has not been systematically investigated. Therefore, we took advantage of our novel model of pediatric ischemic stroke to directly compare plasticity and repair following stroke in the juvenile and adult mouse. Extracellular field recordings of CA1 neurons were performed in acute hippocampal slices prepared at 24 hrs, 7 or30 days after recovery from middle cerebral artery occlusion (MCAO) and compared to sham control mice. Our data shows that in adult mice, hippocampal long-term potentiation (LTP) is impaired as early as 24 hrs after stroke induced by the MCAO and remains impaired for at least 30 days in both the ipsilateral and contralateral, non-injured hemisphere. However, in pediatric mice, LTP is impaired as early as 24 hrs after MCAO and remained impaired in the ipsilateral side 7 days after MCAO, but recovered in the contralateral side at 7 days after MCAO. At 30 day, pediatric mice display a full recovery of synaptic function in both hemispheres. Furthermore, significant experimental data is emerging demonstrating an imbalance between inhibitory and excitatory cortical pathways after ischemic stroke, suggesting that reducing GABAergic inhibitory transmission enhances recovery. To test this, hippocampal slices were incubated in aCSF+L655,708 (100nM), an inverse agonist selective for α5 subunit-containing GABAA receptors, throughout the duration of the experiment. Our data shows that synaptic plasticity was rescued in pediatric mice 7 days following MCAO and 30 days in adult mice. Overall, the present study demonstrates that transient focal ischemia causes functional impairment in the hippocampus at various time points after MCAO and that excessive GABA activity may contribute to impaired synaptic function following ischemic injury, thus inhibition of specific GABA activity may provide a new therapeutic approach to improve functional recovery after stroke.

Disclosures: **J.E. Orfila:** None. **H. Grewal:** None. **T. Shimizu:** None. **R.T. Traystman:** None. **P.S. Herson:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.03/D36

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS081786

Title: Long lasting changes in metabolic efficiency occur during long term potentiation in hippocampal neurons

Authors: **C. M. PEQUIGNOT**, S. SACCHETTI, H.-A. PARK, C. WEISS, P. LICZNERSKI, *E. A. JONAS
Yale Univ. Sch. Med., New Haven, CT

Abstract: Long-term potentiation (LTP) and depression (LTD) are the mechanisms by which neurons modulate their inherent plasticity and provide for the storage and recovery of memories in mammalian brain. Although extensive research has been done regarding the changes in AMPA receptor trafficking that occur in neurons undergoing LTP, less is known about any underlying metabolic changes that may be required. Previous work has indicated that synaptic plasticity is correlated with changes in metabolic efficiency, and that the mitochondrial ion channel protein Bcl-xL may instigate that change due to its association with F1FoATP synthase. Here, we demonstrate that Bcl-xL is responsible for the elevation in ATP levels at synapse-specific sites in hippocampal neurons over the long term after high frequency stimulation in isolated hippocampal neurons. Utilizing FRET imaging techniques, we found that ATP levels remain elevated for up to 45 minutes at synapses after brief glycine stimulation. We found that ATP elevation was dependent on Bcl-xL because it was inhibited by a small molecule Bcl-xL inhibitor, the anti-cancer drug ABT-737. Studies with ABT-737 revealed neurons with ATP levels globally lower than control neurons and not limited to synapse specific sites. In future directions, we will test if Bcl-xL is responsible for driving the change in metabolic efficiency underlying LTP by its long term binding to the F1FoATP synthase after the stimulus has taken place. Our study also suggests that while only certain synaptic sites may undergo LTP in normal physiological conditions, inhibition of Bcl-xL leads to significantly lower ATP in the whole neuron, implicating Bcl-xL as not only essential to synapses experiencing LTP but also the

maintenance of synapses against LTD. Our study places Bcl-xL at the center of metabolism and synaptic plasticity.

Disclosures: C.M. Pequignot: None. **E.A. Jonas:** None. **S. Sacchetti:** None. **H. Park:** None. **P. Licznerski:** None. **C. Weiss:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.04/D37

Topic: B.08. Synaptic Plasticity

Title: CBB68 protects against Aβ oligomer-induced deficits in synaptic function

Authors: R. JEGGO[1], J.-S. WALCZAK[2], A. D. WHYMENT[1], I. VEREYKEN[3], A. TEPPER[3], G. SCHEEFHALS[3], *D. SPANSWICK[1,4,5]
[1]NeuroSolutions Ltd., Coventry, United Kingdom; [2]Cerebrasol Ltd., Montreal, QC, Canada; [3]Crossbeta Biosci., Utrecht, Netherlands; [4]Dept. of Physiol., Monash Univ., Melbourne, Australia; [5]Med. Sch., Univ. of Warwick, Coventry, United Kingdom

Abstract: The transformation of beta-amyloid (Aβ) into soluble, synaptotoxic, prefibrillar oligomers is a major pathogenic event underlying the neuropathology of Alzheimer's disease. Preventing the toxic effects of Aβ assemblies is therapeutically attractive because these events are believed to be exclusively pathogenic. We have investigated the effects of CBB68, a proprietary compound of Crossbeta Biosciences, on deficits in long-term potentiation (LTP) induced by intracerebroventricular (ICV) administration of stabilized oligomers of Aβ. The stabilization is a unique and proprietary technology of Crossbeta Biosciences. Male Sprague-Dawley rats were anaesthetized with urethane and cannulae inserted into the femoral artery and vein for monitoring blood pressure and administering substances (IV), respectively. A craniotomoy was performed and a cannula descended to the left lateral ventricle for ICV administration of Aβ oligomers. Stimulating and recording electrodes were descended to the *stratum radiatum* and *stratum pyramidale* of the CA1 region of the ipsilateral hippocampus for the identification, optimization and monitoring of population spike (PS) activity superimposed on field excitatory postsynaptic potentials. Animals were treated IV with either vehicle or CBB68 before ICV administration of Aβ or Aβ vehicle, followed 60 min later by the induction of LTP using theta-burst stimulation (TBS). PS activity was monitored for a further 90 min. LTP magnitude was derived from the change in PS amplitude pre- and post-TBS and compared

between treatment cohorts. In vehicle treated animals, ICV administration of stabilized oligomeric Aβ induced a significant deficit in LTP compared to those animals injected ICV with Aβ vehicle, with control LTP amounting to 211.5 ± 5.0% of baseline versus 144.6 ± 2.5% after Aβ, 90 min post-TBS. In CBB68 treated animals receiving vehicle ICV, there was a trend for slightly enhanced LTP of 217.9 ± 4.9% of baseline at 90 min, but this did not reach significance compared to control animals. However, CBB68 treatment did partially and significantly prevent the deficits induced by ICV administration of stabilized oligomeric Aβ with 90 min LTP of 182.4 ± 7.8% of baseline, compared to IV vehicle treated animals injected with Aβ (144.6 ± 2.5%). The present data demonstrate the *in vivo* functionality of these stabilized Aβ oligomers in inducing deficits in LTP in the anaesthetized rat hippocampus. They also demonstrate that the novel Crossbeta Biosciences compound CBB68 is centrally penetrant and protects against this Aβ-induced neurotoxicity in an electrophysiological model of learning and memory that is relevant to Alzheimer's disease.

Disclosures: **R. Jeggo:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd. **J. Walczak:** A. Employment/Salary (full or part-time):; Cerebrasol Ltd. **A.D. Whyment:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd. **I. Vereyken:** A. Employment/Salary (full or part-time):; Crossbeta Biosciences. **A. Tepper:** A. Employment/Salary (full or part-time):; Crossbeta Biosciences. **G. Scheefhals:** A. Employment/Salary (full or part-time):; Crossbeta Biosciences. **D. Spanswick:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd..

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.05/D38

Topic: B.08. Synaptic Plasticity

Support: Russian Federation governmental grant No. 11.G34.31.0012

Neuron EraNet TargetECM

Title: Enzymatic removal of chondroitin sulfates modulates neuronal excitability and synaptic plasticity in the hippocampal CA1 region

Authors: ***Y. DEMBITSKAYA**[1], I. SONG[2], M. DORONIN[1], A. DITYATEV[2], A. SEMYANOV[1]

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Abstract: Extracellular matrix (ECM) molecules occupy the space between neurons and glia in the brain and form conspicuous perineuronal nets. Chondroitin sulfate proteoglycans are major components of the ECM involved in synaptic plasticity: however, precise mechanisms of such involvement remain unclear. Using whole-cell recordings in hippocampal slices from male mice (P28-35), we found that acute enzymatic removal of chondroitin sulfates by chondroitinase ABC (ChABC) produced no significant effects on the basal efficiency of either inhibitory or excitatory synaptic transmission, but decreased the number of action potentials (APs) during theta-burst stimulation and impaired theta-burst induced LTP in CA3-CA1 synapses. Consistent with decreased cell excitability ChABC treatment decreased the number of APs in response to somatic current injections. Similar changes were detected in brevican deficient mice, suggesting that brevican is one of the carriers of chondroitin sulfates modulating neuronal excitability. In addition, ChABC treatment increased afterburst hyperpolarization in CA1 pyramidal neurons. Both the decrease in cell excitability and the increase in aftreburst hyperpolarization were prevented by 100 nM apamin, a blocker of the SK family of Ca^{2+} activated K^{+} channels, suggesting up-regulation of SK channel activity in ChABC treated slices. Notably, LTP recorded in the presence of apamin after ChABC treatment was enhanced as compared to slices, which were not treated with ChABC. However, the number of APs during theta-burst stimulation in the presence of apamin was not different between control and ChABC treated slices. This enhancement of LTP was suppressed by 10 μM Y-27632, a ROCK kinase inhibitor, suggesting modulation of RhoA signaling pathway by ChABC treatment. Our findings demonstrate that removal of chondroitin sulfates has a complex effect on synaptic plasticity in the CA1 region: the enhancement of LTP through the RhoA signaling pathway is masked by upregulation of SK channel activity. The later can serve as a protective mechanism that prevents overexcitation of neuronal network due to enhanced LTP.

Disclosures: **Y. Dembitskaya:** 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.; Supported by the Russian Federation governmental grant No. 11.G34.31.0012Neuron EraNet TargetECM., Supported by Neuron EraNet TargetECM.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Renato Frischknecht and Constanze Seidenbecher. **I. Song:** None. **M. Doronin:** None. **A. Dityatev:** None. **A. Semyanov:** 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.; Supported by the Russian Federation governmental grant No. 11.G34.31.0012, Supported by Neuron EraNet TargetECM.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Renato Frischknecht and Constanze Seidenbecher.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.06/D39

Topic: B.08. Synaptic Plasticity

Support: CIHR

Title: Post-synaptic long-term potentiation of gaba synapses in the oval bed nucleus stria terminalis

Authors: *E. HAWKEN, E. C. DUMONT
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Abstract: Neural circuits consist of highly dynamic networks of excitatory and inhibitory neurons. While synaptic plasticity at excitatory synapses throughout the brain is well-established, synaptic plasticity of inhibitory synapses is a far less characterized phenomenon. The bed nucleus of the stria terminalis (BNST) is a structure known to be an interface between homeostatic neuro-regulation and circuits mediating higher cognitive processes. We have recently shown that changes in synaptic plasticity in the oval nucleus (ovBNST) is significantly correlated with drug taking behaviors. Thus, the ovBNST serves as a region of interest for studying the role of plasticity, specifically, that of plasticity at inhibitory synapses. Using whole-cell patch clamping in the slice, low frequency stimulation (LFS; 1 Hz 900 pulses) at inhibitory synapses in the ovBNST produced long-term potentiation (LTP) of GABAa inhibitory post-synaptic currents (IPSC). Twenty-minutes following LFS, IPSCs increased on average 173% ± 77% (SE) above baseline values and was sustained and sometimes continued to increase for up to 60 minutes post-induction (n=9/8). This effect appears to be post-synaptically mediated as there was no change in paired-pulse ratios (0.9± 0.04:1.1 ± 0.09, pre ± SE:post ± SE) and no significant change in the coefficient of variation (1/CV2 pre ± SE:post ± SE, 26.8 ± 8.2: 24.3 ± 6.4). The ubiquity of this phenomenon in the brain has yet to be determined.

Disclosures: E. Hawken: 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.; Canadian Institute of Health Research. **E.C. Dumont:** 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.; Canadian Institute of Health Research.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.07/D40

Topic: B.08. Synaptic Plasticity

Support: NIH grant NS030549 to IM

Title: Synaptic spill-over of LTP in old animals through excitatory GABAergic transmission

Authors: *G. C. FAAS[1], I. FERANDO[1], I. MODY[2]
[1]Neurol., [2]Neurol. and Physiol., UCLA, Los Angeles, CA

Abstract: Age-related neurological disorders correlate with poor memory performance, but few studies have examined synaptic plasticity in mice at the end of their life span. We present new data about our ongoing LTP study in brain slices of old mice (21-25 months). We earlier reported that fEPSPs in the hippocampal CA1 area (evoked by Schaffer collateral stimulation) were significantly more potentiated >20 min after theta burst stimulation (TBS) in slices of old C57BL6/J mice (old-slices), at 30-35 min after TBS the slope of the line as quantified by a novel regression analysis of LTP potentiated by 173±37% (±SD, n=30) in contrast to slices of adult mice (adult-slices, 11-17 weeks old, +62±7%, n=15). Furthermore, pharmacological experiments with $GABA_AR$ modulators indicated that an excitatory switch in GABAergic transmission took place in old-slices, which was responsible for the added potentiation during the maintenance phase of LTP. Upon further analysis we now show that the time course of LTP in 9/30 (30%) old- slices is similar to that seen in 15/15 (100%) adult-slices showing a large post-tetanic potentiation (PTP, t=0-5 min, 115±6%) reduced within 20 minutes to a smaller steady potentiation (t=30-35 min, +62±7%). In 21/30 (70%) old-slices we found a markedly different time course where the potentiation after PTP remains steady or increases (+210±65%). In 3/4 adult-slices the KCC2 blocker VU-0240551 (10μM) converted the time course of LTP to that seen in the majority of old-slices. Thus a switch to an excitatory GABAergic transmission due to a shift in E_{Cl} may be responsible for the GABAergic component of LTP in old-slices. It has been well established that excitatory LTP is highly specific so that LTP is only induced in the pathways that receive the LTP inducing stimulation, while neighboring synapses remain unchanged. However, GABAergic transmission via synapses on spine necks and dendritic shafts is less precise and a depolarization or Ca^{2+} entry generated by such synapses might spill over to neighboring synapses. To address this synapse specificity of LTP we stimulated two distinct afferent fiber tracts and gave only one a TBS. In 3/3 of adult-slices, potentiation did not occur in the pathway that did not receive the TBS. In contrast, 8/10 old-slices showed a steady increasing

potentiation (t=35 min, +45±16%) of the pathway that did not directly receive TBS , indicating that some component of potentiation in old-slices spills over to neighboring inputs. We conclude that in old animals GABAergic transmission can significantly contribute to LTP and that this contribution makes the overall potentiation less synapse specific which likely interferes with memory formation.

Disclosures: **G.C. Faas:** None. **I. Ferando:** None. **I. Mody:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: NIMH K01 Grant

NIMH R01 Grant

Title: Cell type-specific GABAergic plasticity in the prefrontal cortex

Authors: ***C. Q. CHIU**, M. J. HIGLEY
Dept. of Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Neurons communicate via excitatory glutamatergic and inhibitory GABAergic synapses. The interplay between excitation and inhibition not only shapes spike output but also regulates activity-dependent changes in synaptic efficacy. Indeed, several forms of GABAergic plasticity depend on the activation of glutamatergic receptors. However, in most cases, the underlying molecular mechanisms and cellular specificity are poorly understood. This information is vital for the understanding of how plasticity of GABAergic synapses impacts neural circuit function. Using cell type-specific optogenetic stimulation in mouse prefrontal cortical slices, we studied the capacity of GABAergic synapses formed by different interneuron subtypes to exhibit activity-dependent plasticity. Brief activation of NMDA-type glutamate receptors (NMDARs) produced an increase in GABAergic inhibition mediated by somatostatin-positive (SOM) but not parvalbumin-positive (PV) nor vasoactive intestinal peptide-positive (VIP) interneurons. Potentiation of SOM-mediated inhibition (SOM-iLTP) requires postsynaptic activation of NMDARs, a rise in intracellular calcium and activation of CaMKIIa. Similar potentiation of dendritic inhibition evoked by optical uncaging of GABA indicates that SOM-

iLTP is expressed postsynaptically. Our results indicate a crucial role for SOM-iLTP in regulating dendritic signaling and circuit activity within the prefrontal cortex.

Disclosures: C.Q. Chiu: None. **M.J. Higley:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: SFB779/B06

CBBS FKZ1211080005

Title: Presynaptic or BDNF-dependent postsynaptic STDP Expression relies on postsynaptic action potential pattern during STDP induction

Authors: E. EDELMANN[1], P. LICHTENECKER[1], *T. BRIGADSKI[1,2], V. LESSMANN[1,2]
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Abstract: Long-term potentiation (LTP) relying on repeated precisely timed firing of action potentials in pre- and postsynaptic neurons, is a physiologically relevant type of synaptic plasticity. The resulting plasticity is called timing-dependent (t-)LTP. These activity patterns are considered to represent a cellular correlate of memory formation. While brain-derived neurotrophic factor (BDNF) is known to regulate LTP in the CA1 area of the hippocampus upon high frequency stimulation, the signaling mechanisms mediating the physiologically more relevant t-LTP, have remained elusive. Using patch clamp electrophysiology we now investigated different forms of hippocampal spike timing-dependent plasticity (STDP), and identified postsynaptic and presynaptic types of t-LTP, which recruit either BDNF-dependent or -independent signaling mechanisms. Furthermore, we determined patterns of electrical activity inducing release of BDNF from CA1 hippocampal neurons which underlie the BDNF-dependent t-LTP. To analyze an involvement of endogenous BDNF in STDP in hippocampal CA1 region, we used distinct low frequency pairing protocols with different numbers of postsynaptic stimuli and repetitions. Short positive spike timings led to t-LTP for both paradigms (1EPSP/1AP and 1EPSP/4AP). However, the LTP expression mechanism differed between the paradigms. While

the 1EPSP/1AP paradigm recruited a BDNF/TrkB-independent pathway, chronic and acute depletion of BDNF impaired t-LTP induced by the 1EPSP/4AP paradigm. Accordingly, scavenging of extracellular BDNF with TrkB-Fc, selectively impaired t-LTP induced by the 1EPSP/4AP protocol. Pre- vs. postsynaptic changes after t-LTP induction were explored and indicate presynaptic LTP expression for the BDNF-insensitive protocol, but postsynaptic expression for the BDNF-dependent 1EPSP/4AP paradigm. As revealed by inhibition of t-LTP by application of k252a via the patch pipette, postsynaptic TrkB signaling was required for BDNF-dependent t-LTP. Furthermore, postsynaptic release of endogenous BDNF (induced by backpropagating APs in CA1 pyramidal neurons) paired with synaptic stimulation, induced plasticity that occluded BDNF-dependent t-LTP. Fluorescence microscopy of BDNF-GFP release confirmed postsynaptic BDNF secretion under these stimulation conditions. Together, our data suggest that expression of t-LTP in CA1 of the hippocampus is regulated in a stimulus dependent manner, with 1EPSP/4AP pairing eliciting activity-dependent secretion of BDNF from postsynaptic CA1 neurons and postsynaptic t-LTP expression, while 1EPSP/1AP induced t-LTP proceeds by a BDNF-independent mechanism.

Disclosures: E. Edelmann: None. **P. Lichtenecker:** None. **T. Brigadski:** None. **V. Lessmann:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.10/D43

Topic: B.08. Synaptic Plasticity

Support: ERC Grant 268 689

Swiss Government Excellence Scholarship

Title: A cellular and synaptic level model for reconsolidation

Authors: *D. B. KASTNER[1], T. SCHWALGER[2], L. ZIEGLER[2], W. GERSTNER[2]
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Abstract: Reconsolidation, the process by which previously learned memories become susceptible to perturbation and require renewed stabilization, reflects the dynamic nature of memory. Reconsolidation has largely been studied at the behavioral level, providing insight into

its molecular components, but the cellular and computational features of the phenomenon remain unclear. Since the molecular machinery of reconsolidation is distinct from that of the preliminary storage process of consolidation, we developed a model of reconsolidation by extending a previous model of synaptic consolidation. The consolidation model captures the changes in synaptic strength by modeling synapses onto a cell with three interacting bistable equations. The three equations reflect the weight of the synapse, its tagged state, and whether or not it is consolidated. To capture reconsolidation we added a shared pool of molecules that bind to the synapses and stabilize the consolidated state. The unbinding of the molecules is activity dependent, causing a destabilization of the consolidated state exclusively when the neuron is stimulated, mimicking the exclusive loss of a memory at the behavioral level when memory reactivation is coupled with protein synthesis blockade, but not when protein synthesis blockade occurs by itself. Over time these molecules build up a reservoir that can withstand protein synthesis blockage, linking the size and dynamics of the reservoir to the boundary conditions of reconsolidation--situations when reconsolidation does or does not occur. The model replicates hippocampus slice based experiments thought to potentially underlie reconsolidation, firmly establishing those experiments as cellular substrates for reconsolidation. Furthermore we developed a theoretically compact description of the model that allows for an exploration of the parameter space of reconsolidation, and an exact description of the boundary conditions of the model. With such a model we can explore the computational nature of reconsolidation, providing for a better understanding of the dynamic nature of memory.

Disclosures: D.B. Kastner: None. **T. Schwalger:** None. **L. Ziegler:** None. **W. Gerstner:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.11/D44

Topic: B.08. Synaptic Plasticity

Support: NIH MH049159

Cure Alzheimer’s Foundation

Title: Engineering a memory with LTD and LTP

Authors: *S. NABAVI
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Abstract: It has been hypothesized that memories are encoded by modification of synaptic strengths through cellular mechanisms such as long-term potentiation (LTP) and long-term depression (LTD). However the causal link between these synaptic processes and memory has been difficult to demonstrate. Here we show that fear conditioning, a type of associative memory, can be inactivated and reactivated by LTD and LTP, respectively. We begin by conditioning an animal to associate a foot-shock with optogenetic stimulation of auditory inputs targeting the amygdala, a brain region known to be essential for fear conditioning. Subsequent optogenetic delivery of LTD conditioning to the auditory input inactivates memory of the shock. And finally, subsequent optogenetic delivery of LTP conditioning to the auditory input reactivates memory of the shock. Thus, we have engineered inactivation and reactivation of a memory using LTD and LTP, supporting a causal link between these synaptic processes and memory.

Disclosures: S. Nabavi: None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.12/D45

Topic: B.08. Synaptic Plasticity

Title: Learning induced LTP in mice

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Abstract: Long-term potentiation (LTP) is a process by which the strength of the synaptic connection between neurons increases when the synapse is repeatedly activated. It can be "artificially" triggered by electrical stimulation protocols such as high frequency or theta burst stimulations (TBS). There is now a strong body of evidence demonstrating common molecular mechanisms underlying LTP and long term memory, and LTP is currently the best candidate for a neural correlate of memory. However, only few studies succeeded in showing that LTP actually occurs in the hippocampus during learning. We recorded evoked field potentials (fEPSP) in area CA1 of the hippocampus in freely moving mice before and after a session of

contextual fear conditioning. Here we report that this kind of electrophysiological measurement allows monitoring the "natural" LTP triggered by the training session. Moreover we show that conditioning-induced LTP partially occludes TBS-induced LTP, suggesting that natural and artificial LTP share some mechanisms. This study provides the first electrophysiological evidence of a robust LTP induced by a single session of learning in mice.

Disclosures: J. Remaud: None. **F. Roumier:** None. **S. Pech:** None. **B. Francès:** None. **L. Dahan:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

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Program#/Poster#: 404.13/D46

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01AA018060

ONR grant MURI N00014-10-1-0198

Title: Molecular plasticity signature in hippocampal area CA1

Authors: *J. JEDRZEJEWSKA-SZMEK, A. M. CHAY, K. T. BLACKWELL
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Abstract: Hippocampal plasticity is considered one of the major cellular mechanisms of learning and memory. Molecular mechanisms responsible for long lasting forms of LTP (L-LTP) which last from several hours up to days, are of interest because this form of plasticity resembles long term memory storage. Plasticity inducing protocols vary in their activation of signal transduction pathways and resulting dependence on signaling molecules: train (1 s) of 100 Hz stimulation (HFS) elicits only a short-lasting form of LTP (E-LTP) whereas multiple trains of HFS elicit L-LTP, Bath application of isoprotenerol (ISO) followed by electric stimulation produce L-LTP that differs in their molecular dependence: 3 min of 5 Hz stimulation (ISO + LFS) is protein kinase A (PKA) dependent whereas ISO + HFS is not.To investigate how temporal pattern of synaptic activation determines activation of signaling pathways, we employed a multi-compartmental, stochastic reaction-diffusion model of calcium (Ca) and cyclic adenosine monophosphate (cAMP) activated signaling pathways in CA1 pyramidal neurons. We started with the model presented by Kim et al. (2011) and added stimulative regulatory G protein (Gs) to

inhibitory regulatory G protein (Gi) switching (Havekes et al., 2012). We simulated Ca influx and β-adrenergic receptor stimulation produced by eight commonly used plasticity induction paradigms. We discovered that the following plasticity signature can explain the direction of plasticity: a combination of PKA, phosphatatases, and Ca2+/calmodulin-dependent protein kinase II (CaMKII) phosphorylation in the spine may act to "tag" the spine. Specifically, the spine signature is proportional to the sum of CaMKII and PKA activity divided by the phosphatase activity. Three regimes leading to LTP, no change, and long term depression are visible. A combination of PKA, pCaMKII, exchange protein activated by cAMP (Epac) and βγ subunit of Gi (Giβγ), in the dendrite may act to initiate production of plasticity related proteins. Though 1 train of HFS produces a spine signature of LTP, it does not produce a dendritic signature for protein synthesis initiation, explaining why 1 train of HFS only elicits E-LTP. Furthermore, Epac activation is the highest with ISO + HFS, explaining why this paradigm produces PKA independent, but cAMP dependent L-LTP. The molecules required for the dendritic signature are related by their downstream target extracellular signal-regulated kinase (ERK) which is implicated in learning, memory and plasticity. The combination of spine signature and dendritic signature are required to predict the outcome and molecular dependence of synaptic plasticity protocols.

Disclosures: J. Jedrzejewska-Szmek: None. **A.M. Chay:** None. **K.T. Blackwell:** None.

Poster

404. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 404.14/D47

Topic: B.08. Synaptic Plasticity

Support: NINDS Intramural

Title: Incorporation of Calcium-Permeable AMPA receptors at silent synapses during hippocampal long-term potentiation

Authors: *J.-C. RAH[1], D. MORITA[2], J. T. R. ISAAC[2]
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Abstract: Despite decades of study, the mechanisms by which synapses express the increase in strength during long-term potentiation (LTP) remain an area of intense interest. Here, we have studied how AMPA receptor subunit composition changes during the early phases of hippocampal LTP in CA1 pyramidal neurons. We studied LTP at silent synapses that initially lack AMPA receptors, but contain NMDA receptors. We show that strongly inwardly rectifying AMPA receptors are initially incorporated at silent synapses during LTP and are then subsequently replaced by non-rectifying AMPA receptors. These findings suggest that silent synapses initially incorporate GluA2-lacking, calcium-permeable AMPA receptors during LTP that are then replaced by GluA2-containing calcium-impermeable receptors. We also show that LTP consolidation at CA1 synapses requires a rise in intracellular calcium concentration during the early phase of expression, indicating that calcium influx through the GluA2-lacking AMPA receptors drives their replacement by GluA2-containing receptors during LTP consolidation. Taken together with previous studies in hippocampus and in other brain regions, these findings suggest that a common mechanism for the expression of activity-dependent glutamatergic synaptic plasticity involves the regulation of GluA2-subunit composition and highlights a critical role for silent synapses in this process.

Disclosures: J. Rah: None. **D. Morita:** None. **J.T.R. Isaac:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.01/D48

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG042804

Title: Neurobiological role of a novel microRNA regulating amyloid precursor protein (APP) expression via interaction with iron regulatory protein-1 (IRP-1): Implication in Alzheimer disease (AD)

Authors: *J. M. LONG[1], N. CHOPRA[2], J. T. ROGERS[3], B. RAY[2], N. H. GREIG[4], K. SAMBAMURTI[5], D. K. LAHIRI[2]
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Abstract: Inflammation and metals play critical roles in AD. The finding that alleles in the hemochromatosis gene advance the onset of AD sparked research in the metallobiology of AD. The presence of an Iron-Responsive Element (IRE) in the 5'UTR of the APP transcript (APP 5'UTR) supports APP a metaloprotein. Iron, copper and zinc were shown to accelerate the aggregation of the amyloid-β (Aβ) peptide and enhance metal catalyzed oxidative stress associated with amyloid plaques, the central hallmark of AD. The participation of metals in plaque formation and of metal-dependent translation of APP mRNA support chelators as a major therapeutic strategy for AD (Bandyopadhyay et al-2013). APP is regulated post-transcriptionally with changes in iron homeostasis. In the absence of iron, IRP1 binds to an IRE in the APP 5'-UTR and inhibits translation. MicroRNAs (miRNAs) also post-transcriptionally regulate APP expression. Indeed, miR-101 and miR-153 negatively regulate APP expression via the APP 3'-UTR (Long, Ray & Lahiri- 2012; 2014). Here we report that miR-346 stimulates APP expression via the APP 5'-UTR at a site that overlaps with the IRE. The miRanda algorithm predicted a miR-346 target site in APP 5'-UTR that overlapped a known IRE. Reporter assays in HeLa cells transfected with miR-346 mimic and APP 5'-UTR reporter construct revealed that miR-346 significantly stimulated reporter expression via the APP 5'-UTR. With miR-346 transfection, endogenous APP levels were also significantly increased vs. controls, an effect reversed by target protectors confirming the site of interaction. APP mRNA levels were unchanged suggesting a post-transcriptional mechanism. The stimulatory effect of miR-346 on APP was significantly attenuated when AGO2 or IRP1 was knocked down by siRNA. Thus, miR-346 apparently requires the presence of AGO2 to stimulate APP expression and mediates its effect by interfering with IRP1's binding to the IRE sequence in the APP 5'-UTR. miR-346 was successfully transfected into primary human fetal brain mixed cultures as described (Long et al-2014), but miR-346 transfection did not stimulate expression under standard culture conditions. However, APP levels were stimulated by miR-346 transfection following iron chelation by deferroxamine, a condition that would enhance IRP binding to the IRE. This is consistent with miR-346 interfering with IRP1 binding. Therefore, miR-346 may represent a novel drug target in AD, and miR-346 inhibition should then synergize with iron chelation strategies as an AD treatment.

Disclosures: **J.M. Long:** None. **N. Chopra:** None. **J.T. Rogers:** None. **B. Ray:** None. **N.H. Greig:** None. **K. Sambamurti:** None. **D.K. Lahiri:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.02/D49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS057295-01

Drexel University

Title: Calpain mediates degradation of novel APP C-terminal fragments

Authors: *H. WANG[1,2], A. SAUNDERS[1]
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Abstract: Alzheimer's disease (AD) is a progressive neuro-degenerative disease. One of its hallmarks is the deposition of extracellular plaques, composed of amyloid β (Aβ). Aβ is a small peptide generated from proteolytic processing of its precursor, amyloid precursor protein (APP). The process of APP proteolysis and the generation of Aβ have been studied extensively. Canonical APP proteolysis occurs via α-/ β- and γ-secretases. By inhibiting protein degradation systems, including cathepsin, calpain and the proteasome, we have identified hitherto undocumented APP fragments. These novel fragments are not a result of apoptosis induced proteolysis. Utilizing pharmacological approaches suggests that calpain inhibition is likely responsible for the accumulation of the observed novel APP fragments. This indicates that besides canonical processing by α-/ β- and γ-secretases, APP can be processed by yet unidentified mechanism, and the resulting products are degraded via protein degradation, likely calpain.

Disclosures: H. Wang: None. **A. Saunders:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.03/D50

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: O-glcnacylation of app by justicidin a reduces abeta secretion

Authors: *Y. CHUN[1], Y. CHO[1], O. KWON[1], J. KIM[2], H. YANG[2], S. CHUNG[1]
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Abstract: β-amyloid precursor protein (APP) is transported to the plasma membrane, where it is sequentially cleaved by α-secretase and γ-secretase. This pathway is called non-amyloidogenic pathway, since it precludes the production of hydrophobic β-amyloid peptide (Aβ), the main culprit of Alzheimer's disease (AD). Alternatively, once APP undergoes clathrin-dependent endocytosis, it can be sequentially cleaved by β-secretase and γ-secretase at endosomes, producing Aβ (amyloidogenic pathway). O-GlcNAcylation is a novel type of O-linked glycosylation attaching the monosaccharide β-N-acetylglucosamine (GlcNAc) to serine and threonine residues. Recently, we found that O-GlcNAcylation of APP increases the non-amyloidogenic processing of APP and decreases the production of Aβ. In this study, we identified specific increase of O-GlcNAcylation of APP by Justicidin A, which is one of lignin derivatives. Justicidin A increased the level of APP in the plasma membrane. We also found that Justicidin A selectively attenuated the endocytosis of APP, but not that of transferrin receptor. The level of sAPPα increased, while the level of sAPPβ and Aβ was concomitantly decreased by Justicidin A. Blocking the clathrin-dependent endocytosis by inhibitor prevented the effect of Justicidin A, suggesting that the effect of Justicidin A on Aβ production was mainly mediated through the decrease of APP endocytosis. These results strongly indicate that O-GlcNAcylation by Justicidin A increases the plasma membrane targeting of APP and selectively decreases endocytosis rate of APP, thereby enhancing non-amyloidogenic processing of APP. Thus, O-GlcNAcylation of APP could be a new novel therapeutic target for AD.

Disclosures: Y. Chun: None. **Y. Cho:** None. **O. Kwon:** None. **J. Kim:** None. **H. Yang:** None. **S. Chung:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.04/D51

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of O-GlcNAcylation on mitochondrial ATP synthase in Alzheimer's disease pathogenesis

Authors: *M. CHA[1], H. CHO[1], S. JIN[1], C. KIM[1], Y. JUNG[2], D. KIM[1], H. CHOI[1], M. KANG[3], J. KIM[1], H. SONG[2], E. YI[3], I. MOOK-JUNG[1]
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Abstract: O-linked β-N-acetylglucosamine (O-GlcNAc) modification of protein, a post-translational modification that links GlcNAc to the hydroxyl group of Ser or Thr residues, is one of the major forms of protein glycosylation affecting various intracellular events. However, the role of O-GlcNAc modification of proteins in the pathogenesis of neurodegenerative disease such as Alzheimer's disease (AD) is poorly understood. ATP synthase is multi-protein complex that synthesizes ATP from ADP and Pi using the proton gradient generated by electron transport chain as a driving force. Since ATP generation is significantly reduced in AD mouse model and Aβ (major causative factor for AD)-treated neuronal cells, ATP synthase might be affected by Aβ or other pathological condition in AD. Since we found ATP synthase is O-GlcNAcyalted and it binds to Aβ directly, we tried to figure out the relationship between O-GlcNAcylation on ATP synthase and their function under AD pathological condition, especially in the presence of Aβ. We will discuss more details about how Aβ reduces ATP synthase activity and what is the role of O-GlcNAcylation on ATP synthase to regulate ATP synthase function.

Disclosures: M. Cha: None. **H. Cho:** None. **S. Jin:** None. **C. Kim:** None. **Y. Jung:** None. **D. Kim:** None. **H. Choi:** None. **M. Kang:** None. **J. Kim:** None. **H. Song:** None. **E. Yi:** None. **I. Mook-Jung:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.05/D52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: New Zealand Health Research Council

Title: Secreted amyloid precursor protein-alpha (sAPPα) modulates long-term potentiation, induces metaplasticity and regulates glutamate receptor localization in rat hippocampus

Authors: *K. D. PARFITT[1,2,3], B. G. MOCKETT[2,3], D. GUEVREMONT[3,4], K. BOURNE[3,5], W. P. TATE[3,5], J. M. WILLIAMS[3,4], W. C. ABRAHAM[2,3]

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Abstract: Secreted amyloid precursor protein-alpha (sAPPα), a peptide derived from the same parent molecule as amyloid-ß (Aß), has been shown to enhance synaptic plasticity in rats and in an Alzheimer's mouse model, although the mechanisms of its wide ranging effects are not well understood. Its effects on metaplasticity have not yet been explored and its action on glutamate receptor trafficking, a mechanism that supports long-term potentiation (LTP), is unknown. Here we (i) investigated the effect of sAPPα delivered prior to versus at the time of LTP induction, and (ii) determined whether sAPPα alone can regulate NMDA and AMPA receptor localization. Slices were prepared from adult male Sprague-Dawley rats and allowed to recover for 2 h. Baseline field excitatory postsynaptic potentials (EPSPs) were evoked in area CA1 by electrical stimulation of the Schaffer collateral/commissural pathway, and recorded in stratum radiatum. Bath-applied recombinant sAPPα (1-10 nM) did not affect the initial slope of the EPSPs. Application of mild theta-burst stimulation (TBS, 5 trains (5 Hz) of 5 pulses (100 Hz)) induced post-tetanic potentiation (PTP) but not LTP in untreated slices. In contrast, when applied 20 min before, during and following TBS, sAPPα (10 nM) facilitated both PTP and LTP compared to non-treated controls (responses 1h post-TBS = 139.6 ± 3.3% vs 103 ±1.3% of baseline; $p<0.01$). Lower sAPPα (1 nM) and shorter application (30 min just prior and during TBS) also produced LTP (145.6 ± 3.6% of baseline). To determine whether sAPPα has metaplastic properties, we washed the sAPPα (1 nM, 30 min) out of the bath for 30 min prior to TBS. Under these conditions, LTP was still 149 ± 5% of baseline. To probe underlying mechanisms of this plasticity, we tested whether sAPPα (30 min) affected NMDA or AMPA receptor localization using biotin labelling of cell surface proteins followed by Western blot analysis of the NMDAR subunit GluN1and the AMPAR subunit GluA1. Relative changes in cell surface concentrations in response to sAPPα were determined by comparison with levels in control slices. sAPPα induced significant concentration-dependent biphasic effects on plasma membrane-bound GluN1 and GluA1 expression, with decreases relative to control occurring at low doses and increases occuring at intermediate doses ([GluN1: 0.1 nM, 0.79 ± 0.07, $p<0.01$; 1 nM, 1.32 ± 0.12, $p<0.01$; 100 nM, 0.84 ± 0.19, n.s. GluA1: 0.1 nM, 0.65 ± 0.04, $p<0.001$; 1 nM, 1.55 ± 0.05, $p<0.001$; 100 nM, 1.12 ± 0.26, n.s.). These findings support a fundamental role for sAPPα in regulating synaptic plasticity and raise the possibility that this occurs by regulating NMDA and AMPA receptor localization.

Disclosures: K.D. Parfitt: None. **B.G. Mockett:** None. **D. Guevremont:** None. **K. Bourne:** None. **W.P. Tate:** None. **J.M. Williams:** None. **W.C. Abraham:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.06/D53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: New Zealand Health Research Council

Title: Secreted amyloid precursor protein-α overexpression *in vivo* rescues the long-term potentiation deficit in a mouse model of Alzheimer's disease

Authors: M. F. YAHAYA[1,2], K. D. PARFITT[3,4], B. G. MOCKETT[4], L. SCHODERBÖCK[2], H. E. PEACOCK[5], K. BOURNE[5], W. P. TATE[5], S. M. HUGHES[5], *W. C. ABRAHAM[6,4]
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that mostly affects the elderly. AD is characterised by excessive accumulation of the neurotoxic protein amyloid-β (Aβ). If the mechanism is from enhanced processing at the β,γ site of amyloid precursor protein (APP), there will be a concomitant reduction at the α processing site and thereby production of the neurotrophic and neuroprotective secreted amyloid precursor protein-α (sAPPα). To date there has been limited success in treating established AD cases, research has begun to focus on preventive strategies, inadvertently leaving a gap in combating this disease. We hypothesized that established behavioural and electrophysiological deficits caused by amyloid accumulation could be mitigated or reversed by promoting sAPPα production. To test this, we overexpressed sAPPα bilaterally in the hippocampus of 10-month-old transgenic APPswe/PS1ΔE9 mice using lentiviral gene transfer. These mice develop increased extracellular Aβ deposition and behavioural deficits as early as six months of age. Postulating that sAPPα could ameliorate neurotoxic effects of Aβ, these mice were electrophysiologically tested 3-months post-transduction. Hippocampal slices were prepared and synaptic transmission, paired-pulse facilitation (PPF), paired-pulse recurrent inhibition (PPI) and long-term potentiation (LTP) in area CA1 was examined. Extracellular field recordings revealed no significant differences in basal synaptic efficacy, PPF or PPI between groups. However, the magnitude of LTP measured one hour post-theta burst stimulation in the transgenic mice that received control vector (no sAPPα overexpression) was significantly reduced (136 ± 10%, n=9, $p < 0.05$) compared to litter-matched wild-type controls treated with control vector (171 ± 7%, n=11). LTP in the transgenic mice was rescued by the sAPPα vector (172 ± 7% of baseline, n=11, $p<0.05$). These results

indicate that sAPPα overexpression using lentiviral gene transfer can rescue the deficit in LTP seen in this progressive AD mouse model, which suggests a role for sAPPα in reducing at least some of the neurotoxic effects of Aβ, even after plaque development.

Disclosures: **M.F. Yahaya:** None. **K.D. Parfitt:** None. **B.G. Mockett:** None. **L. Schoderböck:** None. **H.E. Peacock:** None. **K. Bourne:** None. **W.P. Tate:** None. **S.M. Hughes:** None. **W.C. Abraham:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.07/D54

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Activity dependent regulation of APP by Plk2: Novel roles in synaptic plasticity

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Abstract: Synaptic plasticity is the ability of synapses to increase or decrease in strength and important in learning and memory. As a form of synaptic plasticity, homeostatic plasticity is a tuning process of synapse strength up or down in order to counter excessive excitation or inhibition in chronic perturbation of neuronal activity. One of the proteins mediated in the homeostatic plasticity is Plk2, an activity inducible member of the polo family of serine/threonine kinases. It is expressed at low level under basal condition but induced by strong synaptic stimulation such as seizure and picrotoxin (PTX). In this plasticity, AMPA receptor trafficking is regulated by many interacting proteins such as NSF. The function of NSF is to maintain AMPAR at postsynaptic membrane by selectively binding to GluA2 subunit. Our lab showed that Plk2 bound to NSF via a novel binding site of Plk2 and disrupted NSF and GluA2 interaction. Alzheimer disease is the most common neurodegenerative disease, characterized by the formation of neurofibrillary tangles and amyloid plaques. These plaques are composed of amyloid β-protein (Aβ) derived from amyloid precursor protein (APP). Proteolytic cleavage of APP is regulated by proteases. β-secretase cleaves APP to generate a secreted extracellular domain and a membrane tethered-C-terminal fragment (β-CTF). The β-CTF is further cleaved by γ-secretase, which releases Aβ. This amyloidogenic processing of APP appears to be involved in

synaptic plasticity since enhanced synaptic activity generates more Aβ which leads to AMPAR removal from the synapse, which implies a mechanism liking Plk2 effect on activity induced APP processing. In this study, we found that Plk2 was induced by PTX and directly bound to and phosphorylated APP at T668/S675. The phosphorylated APP was internalized and promoted Aβ generation. Therefore, Plk2 expression in APP-SwDI mice temporally increased in the vicinity of plaques whereas transgenic inhibition of Plk2 kinase function reduced amyloid plaque formation and Aβ production *in vivo*. Both AMPA receptors and APP were involved in synaptic plasticity by altering spine formation and density. We also showed that GluA2, not GluA1, was changed by APP expression. Therefore, APP and GluA2 seems to have a potential relationship. Indeed, Plk2 induced two phosphorylation sites in APP is necessary for overactivity-induced loss of surface GluA2. Moreover, surface residence of APP appeared to govern GluA2 maintenance on cell surface. As mechanism of APP and GluA2 co-trafficking, we confirmed that NSF also interact with APP *in vitro* and *in vivo*. Therefore, NSF seems to function as a bridge to connect between APP and GluA2 at the membrane.

Disclosures: **J. Lee:** A. Employment/Salary (full or part-time):; Georgetown University. **Y. Lee:** None. **K. Lee:** None. **R.S. Turner:** None. **H. Hoe:** None. **D.T. Pak:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.08/D55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

The Japan-Korea Basic Scientific Cooperation Program from JSPS

Title: ATBF1 is a novel binding protein of amyloid-β precursor protein that affects amyloid-β production

Authors: ***C. JUNG**[1], K.-O. UHM[2], M.-J. KIM[2], M. KAWAGUCHI[3], H. AKATSU[4], Y. MIURA[5], S. MISUMI[1], E.-K. CHOI[7], Y.-S. KIM[8], M. MICHIKAWA[6], H. HIDA[1]
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Abstract: The amyloid-βpeptides, the major protein component of brain senile plaques in Alzheimer's disease (AD), derived from the proteolytic cleavage of an amyloid precursor protein (APP) by β- and α-secretase. However, the mechanisms mediating APP processing is poorly understood. We previously reported that the increase of a homeotic transcription factor ATBF1 (AT-motif binding factor 1) is shown in the brains of 17-month-old Tg2576 mice compared with wild-type controls, and that Aβ42 increases ATBF1 expression, resulting in cell death in primary rat cortical neurons. To clarify whether ATBF1 expression is involved in the mechanism of cell death in human AD, we first investigated the expression of ATBF1 in human AD brains, and found that ATBF1 levels are increased in the cytoplasm of hippocampal neurons in AD brains compared with non-AD brains. We then investigated how ATBF1 induces the cell death focusing APP processing. Cotransfection of human embryonic kidney (HEK293T) and human neuroblastoma (SH-SY5Y) cells with ATBF1 and APP695 increased steady-state levels of APP via the binding of ATBF1 to the APP cytoplasmic domain (amino acids 666-690), resulting in increased Aβ production and cellular and soluble APP (sAPP) levels without affecting the activity or levels of APP processing enzymes (α-, β- or α-secretase). Conversely, knockdown of endogenous ATBF1 reduced levels of cellular APP, sAPP and Aβ in HEK293 cells overexpressing human APP695. In addition, cotransfected APP with ATBF1 into SH-SY5Y cells seems be accumulated and colocolized on Rab5 and 7, early and late endosomes markers, respectively. Our findings provide insight into the dynamics of APP processing and Aβ production, and suggest that ATBF1 is a novel APP binding protein that might be a suitable therapeutic target for AD.

Disclosures: C. Jung: None. **K. Uhm:** None. **M. Kim:** None. **M. Kawaguchi:** None. **H. Akatsu:** None. **Y. Miura:** None. **M. Michikawa:** None. **E. Choi:** None. **Y. Kim:** None. **H. Hida:** None. **S. Misumi:** None.

Poster

405. APP Function and Processing

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Program#/Poster#: 405.09/D56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS056049

NIH P50 AG008702

NIH 5T32GM007367-38

Title: Amyloid precursor protein (APP) is ubiquitinated at multiple sites in the COOH-terminal domain as a signal for endosomal sorting

Authors: *R. WILLIAMSON[1], Z. M. LASIECKA[2], E. MOREL[2], Z. CHAMOUN[2], C. VETANOVETZ[2], S. A. SMALL[3], G. DI PAOLO[2]
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized clinically by a progressive cognitive decline and neuropathologically by amyloid plaques and neurofibrillary tangles. Amyloid plaques are largely composed of amyloid beta peptide derived from cleavage of its parent protein, amyloid precursor protein (APP). According to the amyloid cascade hypothesis, limiting amyloid beta generation in neurons may help prevent or treat AD. The trafficking of APP and its processing enzymes is closely linked to production of toxic metabolites, such as amyloid beta and the C-terminal fragment beta. Though the subcellular locations where APP processing occurs remain poorly understood, growing evidence suggests that amyloidogenic cleavage occurs in endosomes. Our lab implicated an endosomal sorting pathway for APP that has major implications for its amyloidogenic processing. The molecular basis involves recognition of ubiquitinated APP by components of ESCRT (endosomal sorting complex required for transport) to sort APP from the limiting membrane of endosomes into intraluminal vesicles (ILVs) for eventual lysosomal degradation. Remarkably, our published work showed that ubiquitination and ESCRT-dependent sorting of APP into ILVs alters amyloid beta generation, suggesting that this pathway may be exploited for therapeutic purpose in AD. Because APP ubiquitination is critical for ILV sorting, a fundamental question is the identity of the sites that undergo ubiquitination in APP's C-terminus and the type of ubiquitin modification involved. Our lab originally identified an APP mutant with a triple lysine-to-arginine mutation (K724-726R, termed APP-3R) that is deficient in ubiquitination, leading to mislocalization of APP from the endosome interior to the limiting membrane, and an increase in amyloid beta generation. Here, we dissect the individual contribution of each lysine mutated in the APP-3R mutant, and identify other lysine residues in the APP C-terminus that are deficient in ubiquitination and represent sites that may be critical in APP trafficking and processing. In addition, we characterize an APP missense mutation of the leucine residue adjacent to the APP-3R triple lysine site (L723P), which phenocopies the APP-3R mutant by exhibiting a reduction in APP ubiquitination, mislocalization of endosomal APP, and an increase in amyloid beta. Of particular note, the APP-L723P mutation was first discovered as the cause of early-onset AD in one family. By understanding APP ubiquitination and the endosomal sorting pathway, we expect to uncover new targets and avenues for development of urgently needed AD therapeutics.

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Poster

405. APP Function and Processing

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01AG032432

NIH/NIA R42AG031586

NSFC 81200987

Title: Ectodomain of C-terminal fragment, the destiny switch of β-amyloid precursor protein

Authors: ***J. TAN**[1], S. LI[4], H. HOU[2], J. DENG[5], J. TIAN[3], B. GIUNTA[3], Y. WANG[5], D. SAWMILLER[3], P. SANBERG[3], A. SMITH[3], D. OBREGON[3], T. MORI[6]
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Abstract: Residues located at the ectodomain of β-C-terminal fragment (ECD-βCTF) might be pivotal for wild-type β-amyloid precursor protein (APPwt) processing. In this study, using specific monoclonal antibody recognizing ECD-β-CTF (mAbECD-βCTF), we found that the *in vitro* bindings of mAbECD-βCTF and its F(ab')2 fragment shifts APPwt processing from α- to β-cleavage, as evidenced by elevated accumulation of cell-surface full-length APP and β-CTF together with a marked reduction of sAPPα. This effect was mediated by inhibition of APPwt endocytosis and blocking of ADAM10 mediated α-cleavage. Consistent with these *in vitro* data, intracerebroventricular injection of mAbECD-βCTF markedly increases membrane-associated β-CTF levels in TgAPPwt mice brain. All together these findings suggest that the ECD-β-CTF is critical for APPwt processing and may provide the foundation for a novel family AD therapeutics. Keywords: Alzheimer's disease / amyloid-β precursor protein / amyloid-β / β-C-terminal fragment / ectodomain of β-C-terminal fragment / endocytosis

Disclosures: **J. Tan:** None. **S. Li:** None. **H. Hou:** None. **J. Deng:** None. **J. Tian:** None. **B. Giunta:** None. **Y. Wang:** None. **D. Sawmiller:** None. **P. Sanberg:** None. **A. Smith:** None. **D. Obregon:** None. **T. Mori:** None.

Poster

405. APP Function and Processing

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.11/D58

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Aβ-induced degradation of BMAL1 and CBP leads to circadian rhythm disruption in an Alzheimer's disease mouse model

Authors: ***H. SONG**[1], S. KANG[1], M. MOON[1], H. CHOE[2], D.-H. HAN[3], C. JANG[1], S. CHO[3], K. KIM[2], I. MOOK-JUNG[1]
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Abstract: **Abstract** Circadian rhythm disruption is commonly found in patients with neurodegenerative disease including Alzheimer's disease (AD). AD is an age-related neurological disorder and Aβ is one of major causative molecules in the pathogenesis of AD. Since it is largely unknown whether and how circadian clock molecules affect the circadian rhythm disruption in AD patients, we tried to investigate the link between Aβ and circadian clock molecules such as PER2, BMAL1 and CBP in AD animal model mouse, 5XFAD mice. We found that AD mice showed the increases of both home cage activity and body temperature with clock molecule alteration. To elucidate the molecular mechanism of these changes in AD, Aβ was treated to HT22 cells, hippocampal neuronal cell line. Aβ reduced BMAL1 stability by increase of sumoylation on BMAL1. At the same time, Aβ reduced CBP level and increased N-cadherin cleavage, which is occurred by γ-secretase. Treatment of γ-secretase inhibitors to the cells caused blockade of N-cadherin cleavage, resulting in increase of CBP level, suggesting that Aβ-induced CBP degradation is through the γ-secretase dependent N-Cadherin cleavage. Both BMAL1 and CBP stabilities affected PER2 expression. It suggests a critical role of Aβ in circadian rhythm disruption and implicates the underlying molecular mechanism in the progress of AD pathogenesis. Key words: Alzheimer's disease, Circadian rhythm, Amyloid-beta (Aβ), BMAL1, CBP

Disclosures: **H. Song:** None. **S. Kang:** None. **M. Moon:** None. **H. Choe:** None. **D. Han:** None. **C. Jang:** None. **S. Cho:** None. **K. Kim:** None. **I. Mook-Jung:** None.

Poster

405. APP Function and Processing

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.12/D59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Transcription regulation of the human USP25 gene

Authors: ***B. SONG**, F. CAI, W. SONG
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Abstract: Dysregulation of the ubiquitin proteasome pathway has been implicated in the pathogenesis of neurodegenerative diseases and Down Syndrome. The ubiquitin-specific proteases 25 (USP25) gene spans over 150kb and located in Chr21q11.2. To define the molecular mechanism of USP25 gene transcriptional regulation, we isolated a 2.2-kb 5'UTR of USP25 gene. A series of nested deletions of the 5'UTR fragments were subcloned into a luciferase reporter plasmid pGL3-Basic. HEK293 cells were transfected with the USP25 promoter constructs and luciferase activity was measured to assay its promoter activity. We identified a 104-bp fragment containing the transcription initiation site as the minimal region necessary for USP25 gene promoter activity. Several putative cis-acting elements, such as SP1, HIF and CdxA are found in the 5' flanking region of USP25 gene. Further analysis will reveal which transcription factor regulates the promoter activity of the human USP25 gene. Supported by Canadian Institute of Health Research

Disclosures: **B. Song:** None. **F. Cai:** None. **W. Song:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

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Program#/Poster#: 405.13/D60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA 1 R21 AG041456-01A1

Alzheimer's Drug Discovery Foundation Proposal 280602

Title: Detecting and blocking the intracellular caspase cleavage of amyloid precursor protein as a biomarker of Alzheimer's disease - screening for inhibitors and validation of a new cleavage site-specific antibody that detects APP delta C31

Authors: K. S. POKSAY[1], J. CAMPAGNA[1], P. R. SPILMAN[1], D. KANE[1], R. LIU[2], J. ZIELINSKI[2], M. MULLENIX[2], D. J. SHEFFLER[3], *D. E. BREDESEN[1,4], N. D. P. COSFORD[3], V. JOHN[1,4]
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Abstract: In addition to β-amyloid-containing extracellular plaques and tau-containing intracellular tangles, Alzheimer's Disease (AD) is characterized by neuronal and synaptic loss in specific areas of the brain. One process that likely contributes to this destruction is the intracellular caspase cleavage of the amyloid precursor protein (APP) resulting in the release of the toxic and short-lived C-terminal 31 aa fragment (C31). We previously found that if we block this C-terminal cleavage of APP, cell death as well as many mouse AD phenotypes were prevented. Here, we show methods we have developed and findings we have made using a new D664 (of APP695) cleavage site-specific antibody (APP delta C31) to measure the level of the resulting N-terminal fragment and our progress toward discovering inhibitors of this process. The techniques we have utilized and developed thus far with this APP delta C31 antibody include immunoassays (ELISA and AlphaLISA), immunoblot, and immunoprecipitation. The cell model we use here is Chinese Hamster Ovary cells stably transfected with APP770 (CHO-7W). We are able to generate cleavage at the D739 site (of APP770) by treating the cells with some statins or staurosporine and we are also able to knock down this signal with the pan caspase inhibitor Q-VD-OPh in a dose-dependent manner. AlphaLISA is used as a high-throughput screening tool to identify hits and an APP delta C31 ELISA kit is used to validate these hits. Thus far, we have tested caspase inhibitors and small molecule compound libraries where we have identified several hits that are effective at lowering the APP delta C31 signal in a dose-dependent manner. As further validation of hits, we are using *ex vivo* organotypic hippocampal slice cultures from young I5 mice (overexpressing hAPPwt). Using this model system, we are able to show significant APP delta C31 signal stimulation with cerevastatin and almost complete knockdown with Q-VD-OPh. In addition, we have performed immunoblots to detect APP delta C31 where we demonstrate results similar to those obtained by ELISA as well

as the antibody's specificity for cleaved APP. This antibody is also able to immunoprecipitate APP delta C31. In summary, this new APP delta C31 antibody and ELISA kit have proven to be extremely useful tools for detecting the N-terminal fragment resulting from the intracellular caspase cleavage of APP in cell lysates, organotypic hippocampal slice cultures and CSF. This APP fragment could be a potential biomarker for AD and thus, in addition to the *in vitro* and *ex vivo* testing shown here, these techniques would also be useful for *in vivo* testing of caspase inhibitors and other compounds found effective at inhibiting this cleavage.

Disclosures: K.S. Poksay: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In-kind support (ELISA kits and antibody), Enzo Life Sciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); royalty, receipt of intellectual property rights, Enzo Life Sciences. **J. Campagna:** None. **P.R. Spilman:** None. **D. Kane:** None. **R. Liu:** None. **J. Zielinski:** None. **M. Mullenix:** None. **D.J. Sheffler:** None. **D.E. Bredesen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In-kind support (ELISA kits and antibody), Enzo Life Sciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); royalty, receipt of intellectual property rights, Enzo Life Sciences. **N.D.P. Cosford:** None. **V. John:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); In-kind support (ELISA kits and antibody), Enzo Life Sciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); royalty, receipt of intellectual property rights, Enzo Life Sciences.

Poster

405. APP Function and Processing

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Program#/Poster#: 405.14/D61

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DePaul University Research Council #600757

Title: Modulation of amyloid precursor protein (APP) processing by growth associated protein 43 (GAP-43)

Authors: K. TRACY, A. MILLER, E. SAVAGLIO, *E. M. NORSTROM
Biol. Sci., Depaul Univ., CHICAGO, IL

Abstract: The neurodegenerative disorder Alzheimer's disease is characterized by the accumulation in the brain of the beta-amyloid (Aβ) peptide, which is derived from the proteolytic processing of the amyloid precursor protein (APP). The peptide is released by proteolytic cleavage of APP, first by the β-secretase enzyme and subsequently by the enzyme complex γ-secretase which releases the peptide into the extracellular space. In an alternative processing pathway, APP can first be cleaved by α-secretase activity within the Aβ segment precluding its formation. In this case, subsequent cleavage by γ-secretase releases the non-pathogenic P3 peptide. Thus, cellular proteins that may modulate these processing fates are of great interest. A previous proteomic screen of *in vivo* APP-interacting proteins identified growth associated protein 43 (GAP-43) as a potential APP-interacting protein. Like APP, GAP-43 is present at the presynaptic terminal and in growth cones, and brain levels are decreased in severe Alzheimer's disease. Here, we show that APP and GAP-43 can be co-immunoprecipitated from cultured cells expressing both proteins. Expression of GAP-43 alters APP processing by reducing the generation of C-terminal fragments that result from the activity of α- and β-secretase. These effects are dependent on the palmitoylation of GAP-43 at cysteines 3/4. Mutations of cysteines 3 and 4 to alanine eliminated the effect of GAP-43 on APP processing. Moreover, mutant GAP-43 did not co-immunoprecipitate with APP. These effects were consistent with co-localization experiments by fluorescence microscopy. The effect of GAP-43 phosphorylation at serine was also tested for its effect on APP processing using co-expression in cell culture. These data support a model in which palmitoylation of GAP-43 induces membrane localization in the molecule where efficient interaction with APP can occur to modulate its processing.

Disclosures: K. Tracy: None. **A. Miller:** None. **E.M. Norstrom:** None. **E. Savaglio:** None.

Poster

405. APP Function and Processing

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 405.15/D62

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Aberrant Subcellular Distribution of CREB/PCREB in Alzheimer's disease

Authors: *P. MEMAR ARDESTANI, M. SHAMLOO
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Abstract: Cyclic AMP response element-binding protein (CREB) is a nuclear transcription factor that regulates the expression of genes involved in neuronal survival and cognitive

function. The phosphorylated form of CREB (pCREB) binds to the coactivators, CREB binding protein (CBP) and p300, and facilitates expression of its target genes. Previous studies have reported that CREB-mediated gene expression is impaired in Alzheimer's disease (AD). An impairment in nuclear translocation of phosphorylated proteins has previously been shown in other experimental models of the neurodegenerative diseases such as Parkinson's disease (PD). Here we evaluated cellular distribution of CREB /pCREB in experimental model of the AD. We have shown that CREB and pCREB levels are lower in nuclear fraction of brain lysates from Thy1-APP$^{Lond/Swe}$ mouse model of AD compared with their wild type littermates. Furthermore in primary hippocampal neurons of Thy1-APP$^{Lond/Swe}$ mice, pCREB/CREB is localized to the cytoplasmic fraction while in wild type neurons pCREB/CREB is localized to the nucleus. In conclusion, we have shown an aberrant localization of the CREB/pCREB to cytoplasm in both *in vivo* and *in vitro* models of AD.

Disclosures: P. Memar Ardestani: None. **M. Shamloo:** None.

Poster

405. APP Function and Processing

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Program#/Poster#: 405.16/D63

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Characterizing G-quadruplex mediated regulation of the amyloid precursor protein expression

Authors: *E. M. CRENSHAW[1], B. LEUNG[1], N. SEBASTIAN[1], S. ANSALONI[1], P. BEVILACQUA[2], A. J. SAUNDERS[1]
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Abstract: Alzheimer's disease is an age-related, progressive, neurodegenerative disease, which is the most common form of dementia in the developed world. It is the 6th leading cause of death in the United States and the prevalence of this disease is increasing. Neuropathologically, Alzheimer's disease is defined by the accumulation of the β-amyloid (Aβ) peptides and hyperphosphorylated forms of tau. The accumulation of these species leads to synaptic dysfunction, neuronal loss that eventually results in cognitive decline. Most cases occur after the age of 65 years (late-onset), however there are a small number of cases that occur before the age of 60 years (early-onset). Alzheimer's disease can be caused by genetic changes that result in increased Aβ production. Aβ is produced by the proteolytic cleavage of the Amyloid Precursor

Protein by the β- and γ-secretases. Individuals with Down's syndrome (Trisomy 21) have an additional copy of the APP gene, which is located on chromosome 21, and invariably develop Alzheimer's disease because of increased APP proteolysis and Aβ production. This strongly suggest that dysregulation of APP expression could play a role in Alzheimer's disease pathogenesis. Therefore it is imperative to identify mechanisms underlying the regulation of APP expression. Our lab has recently identified the presence of a Guanine Quadruplex located in the 3'untranslated region of APP. A Guanine Quadruplex is a sequence of guanine repeats that fold into secondary structures which can regulate gene expression. Guanine Quadruplexes are conserved sequences that have been located in DNA as well as in the 5' or 3' untranslated regions and coding sequences of mRNAs. Guanine Quadruplexes are emerging as important regulatory sequences of expression. Using Circular Dichroism, we structurally confirmed the presence of the Guanine Quadruplex in the 3' untranslated region of the Amyloid Precursor Protein; as well as a Luciferase Assay and reporter constructs to demonstrate that the Guanine Quadruplex sequence negatively regulates Amyloid Precursor Protein expression in a post-transcriptional manner.

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Poster

405. APP Function and Processing

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Program#/Poster#: 405.17/D64

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Acute effects of amyloid beta 42 on the GUT enteric nervous System (ENS)

Authors: *H. RABE, A. BRAUN, K.-H. SCHÄFER
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Abstract: Introduction The enteric nervous system (ENS) is one component of the neural control system of the digestive tract. It works in concert with the CNS, integrative pathways that pass through sympathetic ganglia and the gastro-enteropancreatic endocrine system. The ENS mirrors the central nervous system in its inner architecture, the dense neuropil and the variety of individual neuronal subtypes. Therefore, molecules like amyloid beta (Abeta), which influences CNS neurons via its neurotransmitter receptors, have theoretical the ability for direct interactions with ENS neurons. Here we investigated influences of Abeta on the ENS, in particular the

participation of the alpha7 nicotinic acetylcholine receptor (α7nAChR) with electrophysiological methods, calcium-imaging, and GUT motility measurements. Methods and Results Abeta was generated with a transgenic neuroblastoma cell line (SH-SY-5Y). The neuronal activity of dissociated ENS cell cultures was measured with a multi electrode array system (MCS). Counts of action potentials and spikes amplitudes were analysed in control medium, in pre-incubated control medium, in medium containing Abeta peptides, and in Abeta medium with 0.1 μM methyllycaconitine (MLA), respectively. The Abeta42 peptides containing medium strongly reduces firing frequencies in about two thirds of all responding electrodes. Additionally applied MLA moderates the Abeta effect significantly. In calcium imaging experiment with Fluo-4 AM we could confirm a cytotoxic effect of Abeta on short time cultured ENS cells. We observed a strong dose dependent increase of the intracellular calcium concentration when 1 μM and 10 μM Abeta was applied to cells. Furthermore MLA significantly blocked the calcium elevation induced by Abeta. Gastrointestinal motility changes were monitored and evaluated as described by Schreiber (Schreiber et al., 2013). Small intestine with mesenteric arteries were dissected from healthy adult rats. In an organ bath approach the gut segment was perfused with amyloid containing medium and with Abeta medium containing MLA, respectively. The perfusion of the gut with Abeta conditioned media led to a dramatic increase of the smooth muscle tonus, combined with a decrease of the gut diameter. This effect was significantly weaker when the small intestine was perfused with Abeta medium containing 0.1 μM MLA. Conclusion Amyloid beta features strong and direct effects on the electrical activity of ENS cells and on the network activity of the ENS in the GUT. Due to the prohibiting effects of the specific blocker MLA an important part of Abeta effects on the ENS were induced via the α7nACh receptors.

Disclosures: **H. Rabe:** None. **K. Schäfer:** None. **A. Braun:** None.

Poster

405. APP Function and Processing

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS grant R01 NS073512

Title: Endothelin-converting enzymes modulate intracellular Aβ accumulation and aggregation

Authors: ***J. PACHECO-QUINTO**, A. MESZAROS, E. ECKMAN
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Abstract: It is widely accepted that the extensive accumulation of amyloid beta peptide (Aβ) aggregates in the brain, in the form of insoluble amyloid deposits and soluble oligomers, contributes to synaptic dysfunction and neuronal loss in Alzheimer's disease (AD). However, the pathophysiological mechanisms leading to imbalances in Aβ homeostasis and eventual Aβ accumulation and aggregation are not well understood. Of the several physiologically relevant Aβ degrading enzymes, the endothelin-converting enzyme family (ECE) has the unique characteristic of degrading Aβ in compartments where Aβ production takes place. Our work has brought to light that ECEs, rather than participating in the clearance of excess extracellular Aβ, degrade the peptide before secretion, in early and recycling endosomes, and degrade *in situ* a pool of intracellular Aβ produced along the endosomal/lysosomal pathway. In SH-SY5Y cells overexpressing human wild type APP, we found that pharmacological inhibition of endogenous ECEs caused Aβ to accumulate to micromolar levels within late endosomes, and led to the formation of soluble Aβ oligomers. Combining ECE inhibition with other factors known to influence AD pathology such as ApoE, high levels of cholesterol, or disruption of lysosomal function, led to the formation of insoluble Aβ species associated with intracellular lipid rafts. While in undifferentiated cells Aβ was found accumulated in the soma, in polarized/differentiated cells the accumulation of intracellular Aβ was predominantly observed along neurites, suggesting that ECEs may degrade Aβ along the retrograde transport pathway. Using synaptosomal preparations from rat brain, we also obtained evidence of ECE activity in synapses. Secretion of Aβ from these preparations was enhanced when ECEs were inhibited. Based on these results, we propose that ECEs degrade Aβ within at least two types of Aβ-producing vesicles; presynaptic vesicles that discharge Aβ at the synaptic cleft and vesicles of synaptic origin that preserve the ability to generate Aβ during retrograde transport. Failure in ECE activity may enhance synaptic Aβ secretion and accumulation of Aβ along axons and presynaptic ends, in concentration high enough to induce Aβ aggregation. Understanding whether ECE dysfunction represents an early step in AD progression may help to formulate novel therapeutic and prevention strategies.

Disclosures: **J. Pacheco-Quinto:** None. **A. Meszaros:** None. **E. Eckman:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.01/D66

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SHRSP - a valid model for mixed neurodegenerative and vascular pathologies?

Authors: ***S. NIKLASS**[1], C. GARZ[1], C. Z. BUECHE[2], K. G. REYMANN[2,3], H.-J. HEINZE[1,2,3], M. M. M. WILHELMUS[4], S. SCHREIBER[1]
[1]Otto-von-Guericke Univ., Magdeburg, Germany; [2]German Ctr. for Neurodegenerative Dis. (DZNE), Magdeburg, Germany; [3]Leibniz-Institute for Neurobio. (LIN), Magdeburg, Germany; [4]VU Univ. Med. Ctr., Amsterdam, Netherlands

Abstract: Background: In human autopsy studies of the non-demented elderly and Alzheimer's disease small vessel pathology, parenchymal amyloid-ß (Aß) aggregates and accumulations of intraneuronal hyperphosphorylated tau (ptau) and neurofibrillary tangles (NFT) are observed simultaneously. Whether neurodegeneration and cerebral small vessel disease (CSVD) occur independently or whether there is a link between both phenomena is poorly understood; indeed, corresponding non-transgenic animal models are rarely established. Within our study we assessed whether spontaneously hypertensive stroke-prone rats (SHRSP) - a valid model of human CSVD - are suitable to investigate the interplay of mixed neurodegenerative and vascular pathologies. Methods: Brains of 88 SHRSP and 44 Wistar controls (12-44 weeks) with age-related microangiopathic changes were investigated for the existence and expression of the amyloid precursor protein (APP, western blotting, immunohistochemistry), Aß accumulations (HE-staining, Congo red (CR) staining, immunohistochemistry), ptau, (immunohistochemistry) and NFT (silver staining). Pilot studies using Methoxy-X04 (CR derivate, 2-photon-microscopy) were conducted for intravital detection of Aß in SHRSP. Results: SHRSP develop different age-related CSVD stages: blood-brain-barrier (BBB) disturbances (starting at an age of 18 weeks), microbleeds (24 weeks), microthromboses and infarcts (31 weeks). Already at an age of 20 weeks, when CSVD is dominated by BBB breakdown, SHRSP show a significant higher APP expression compared to the control group. Starting at an age of 20 weeks there was a significantly age-dependent incidence of parenchymal Aß depositions in SHRSP compared to controls; contrary cerebral amyloid angiopathy (CAA) was seen in only few animals. In Aß positive SHRSP ptau and NFT were detectable four to six weeks after Aß depositions occurred. Pilot data suggest a possible intravital detectability of perivascular Aß. Conclusions: SHSRP, a non-transgenic experimental model of CSVD, develop temporal neurodegenerative changes in line with the "amyloid cascade hypothesis". SHRSP might therefore serve as suitable model to investigate the interplay between vascular and neurodegenerative pathologies. Further investigations have to shed light on the issue whether APP up-regulation or Aß drainage disturbances drive the amyloid pathology in this model.

Disclosures: S. Niklass: None. **C. Garz:** None. **C.Z. Bueche:** None. **K.G. Reymann:** None. **H. Heinze:** None. **M.M.M. Wilhelmus:** None. **S. Schreiber:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.02/D67

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Impaired cholesterol homeostasis increases the secretion of Aβ peptide in FAD-associated presenilin mutant

Authors: *Y. CHO, O.-H. KWON, Y. CHUN, S. CHUNG
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Abstract: Mutations in presenilin genes (PS1 and PS2) are responsible for the majority of Familial Alzheimer's disease (FAD). PS mutations lead to several key cellular phenotypes, including alterations in proteolysis of β-amyloid precursor protein (APP) and Ca^{2+} entry. We previously showed that the level of phosphatidylinositol-4,5-bisphosphate (PIP2) is down-regulated in FAD mutant cells, which is responsible for the increased secretion of β-amyloid peptide (Aβ) (Landman et al., 2006). It is also reported that PS mutant elevates levels of cholesterol due to the increased expression of CYP51, which plays a critical role for the cholesterol synthesis (Tomboli et al., 2008). Thus, in addition to a diverse array of molecular and cellular functions, PS is involved in the metabolism of both cholesterol and PIP2. In this study, we tested whether there exists functional link between the impaired cholesterol homeostasis and the down-regulation of PIP2 in PS mutant cell. Consistent with previous report, cholesterol level was increased in PS mutant cell compared to PS wild type cell. When PS mutant cell was incubated with CYP51 specific inhibitor, cholesterol level was decreased to that of control cell. In contrast, cholesterol level in PS wild type cell was not changed by CYP51 inhibitor. PIP2 level was increased significantly to that of control cell by CYP51 inhibitor, which suggest that the impaired cholesterol homeostasis caused the down-regulation of PIP2. Consistent with the close relationship between PIP2 level and secretion of Aβ, CYP51 inhibitor decreased Aβ levels from PS mutant cell. These results suggest that cholesterol and PIP2 metabolism is closely linked under the regulation of PS, and that the impaired cholesterol homeostasis is underling mechanism for the increased secretion of Aβ.

Disclosures: **Y. Cho:** None. **O. Kwon:** None. **Y. Chun:** None. **S. Chung:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

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Program#/Poster#: 406.03/D68

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research Grant MOP-84480

Title: Roles of insulin-like growth factor-II receptor and lysosomal enzymes in Alzheimer's disease pathology

Authors: *Y. WANG[1], D. WESTAWAY[2], S. KAR[3]
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Abstract: The insulin-like growth factor-II (IGF-II) receptor is an important regulator of the endosomal-lysosomal (EL) system involved in the transport of newly synthesized lysosomal enzymes cathepsins B and D from the trans-Golgi network to endosomes. Evidence suggests that up-regulation of certain lysosomal enzymes within lysosomes can prevent sub-lethal damage, whereas sustained release of the enzymes into cytosol can induce cell death via cytochrome c release from mitochondria. However, very little is known about functional interrelationship between the IGF-II receptor and lysosomal enzymes and their significance in Alzheimer's disease (AD). Since EL system is critical in the generation of β-amyloid (Aβ) peptides, which play important roles in the degeneration of neurons and development of AD pathology, we hypothesize that release/activation of lysosomal enzymes may participate in Aβ-mediated toxicity and development of AD pathology. We used oligomeric human β-amyloid (Aβ)1-42-induced primary mouse cortical neuronal death model to evaluate the levels/activities and subcellular distributions of IGF-II receptor and lysosomal enzymes i.e. cathepsins B and D during neurodegeneration. In addition, we explored the neuronal death mechanisms using different cell death pathways. Ongoing experiments are also being carried out in the cortex of mutant APP transgenic mice such as TgCRND8 mouse models of AD. We found levels of cathepsins B and D and to some extent the IGF-II receptor were increased with time during the oligomeric Aβ1-42-induced neuronal death. The increased cytosolic release of cathepsins B and D was associated with increased expression of pro-apoptotic molecular markers such as Bcl-2-associated X protein, cytosolic cytochrome c, cleaved caspase 3 and nuclear translocation of apoptosis inducing factor. In parallel, our experiments with transgenic mice overexpressing Aβ also showed increased levels of IGF-II receptor and cathepsins B and D in the vulnerable cortical regions of the brain compared to control mice. These results, taken together, suggest a direct role

for cathepsins B and D in AD pathogenesis. Supported by grant from Canadian Institutes of Health Research.

Disclosures: **Y. Wang:** None. **D. Westaway:** None. **S. Kar:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.04/D69

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS086965

Margaret Q. Landenberger Research Foundation

Alzheimer's Association

Title: Alzheimer's disease transgenic mice exhibit impairments in spatial discrimination that coincide with alterations in hippocampal neurogenesis

Authors: U. TOSI[1], M. S. PYFER[2], A. HAZRA[2], X. ZHANG[2], *J. CHIN[2]
[1]Biol. Basis of Behavior, Univ. of Pennsylvania, Philadelphia, PA; [2]Dept. of Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Alzheimer's disease (AD) is characterized by progressive cognitive deficits and memory loss. Studies in transgenic mouse models of AD can provide insights that are difficult to obtain in human patients. Transgenic mice that overexpress human amyloid precursor protein (APP) carrying mutations linked to familial autosomal dominant forms of AD are often used to assess the role of APP and the Abeta peptides produced from it. Our studies in APP mice aimed to understand how APP and/or Abeta affect cognitive function. We focused on the hippocampus, a brain region that is critical for memory formation and is particularly vulnerable in AD. We developed a novel task to assess spatial discrimination, the ability to distinguish and remember similar patterns or contexts, which is heavily dependent on the hippocampus. Using this task, we found that APP mice exhibit age-dependent deficits in spatial discrimination. Because adult-born neurons in the dentate gyrus of the hippocampus are particularly important for spatial discrimination, we examined the timecourse of alterations in neurogenesis with respect to the development of deficits in spatial discrimination. Our studies demonstrate that alterations in hippocampal neurogenesis develop in concert with deficits in spatial discrimination in APP mice,

suggesting a link between the two. Finally, we found that alterations in Wnt signaling pathways in the hippocampus of APP mice may play an important role in initiating dysregulation of neurogenesis and downstream hippocampal function. Together these results suggest that restoring Wnt signaling may normalize hippocampal neurogenesis and improve cognitive function.

Disclosures: U. Tosi: None. **J. Chin:** None. **A. Hazra:** None. **X. Zhang:** None. **M.S. Pyfer:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG038739

NIH training grant DK7061

Title: The impact of long-term high-fat diet consumption, and diet reversal intervention, on inflammation, learning and memory, and behavior in an Alzheimer's mouse model

Authors: *J. M. WALKER, J. A. KENNARD, S. DIXIT, F. E. HARRISON
Vanderbilt Univ., Nashville, TN

Abstract: Previous research has shown that diabetes type 2 is a risk factor for developing Alzheimer's disease (AD), and emerging research has implicated AD brain dysfunction in the pathogenesis of diabetes. Both diseases processes are associated with increased oxidative stress, inflammation, increased expression of Advanced Glycation End products, and cognitive deficits. While some studies have looked at the effect of the AD state on the development of obesity, very little research has examined the effect of an obese, insulin-resistant state on the development of AD, or the possible therapeutic value of diet reversal interventions on cognition and behavior. The present project was designed to test the hypothesis that obesity and insulin resistance would accelerate AD neuropathology (amyloid levels, inflammatory response, oxidative stress), and cognitive dysfunction in a mouse model of AD. Seventy-two male and female APPSWE/PSEN1deltaE9 transgenic mice and wildtype littermates were used in the present study. Mice were group-housed, and diet interventions (high-fat: 60% kcal fat from lard; low-fat:

10% kcal fat. Research Diets) began at 2-months of age and continued for 4 months. At 6-months of age, mice were tested in a variety of behavioral tasks: open-field, elevated zero maze, y-maze, contextual fear conditioning, and nest building. Fasted blood sugar levels were taken to confirm diabetic state in high fat fed mice. Inflammatory and astrocytic markers (TNF-alpha, IL-1b, IL-6, S100b, GFAP) and RAGE were measured in cortex using western blotting. Amyloid-beta levels in hippocampus were measured using ELISA. After behavioral testing, a subset of mice were switched from a high-fat to a low-fat diet to examine whether diet intervention could ameliorate inflammation, as well as behavioral and cognitive dysfunction. High-fat diet fed mice showed higher fasting blood sugar levels compared to low-fat diet fed mice. Both genotype and high fat diet impacted locomotor activity, with APP/PSEN1 mice traveling greater distances than wildtype mice and hypoactivity seen in high fat fed mice. Given the greater numbers of people reaching older ages and becoming at-risk for AD, and the increasing prevalence of obesity, it is critical to demonstrate both the pathological changes that drive cognitive deficits, and more critically, low-cost interventions that can ameliorate them.

Disclosures: **J.M. Walker:** None. **J.A. Kennard:** None. **S. Dixit:** None. **F.E. Harrison:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant NIRG-08-92033

NIH Grant 1R15AG039008

Title: The effect of progranulin loss on an alzheimer's mouse model

Authors: ***C. VOLLERT**, K. NGO, H. POURZARGHAM, J. L. ERIKSEN
Pharmacol. and Pharmaceut. Sci., Univ. of Houston, Houston, TX

Abstract: Alzheimer’s Disease (AD) is the most common cause of neurodegeneration in patients over the age of 65. Pathological hallmarks of AD include the formation of plaques and neurofibrillary tangles in the brain that lead to gross loss of brain function. Recent evidence has shown that mutations in the human progranulin gene (GRN) are associated with frontotemporal lobar dementia with ubiquitinated TDP-43 inclusions. Current studies suggest that reductions in

progranulin expression have a broad importance for neurodegenerative disease and may represent a possible a risk factor for AD. Despite a growing interest in the role of PGRN in dementia, there are no studies characterizing the behavioral and neuropatholgical effects of PGRN haploinsufficiency in an AD transgenic mouse model. In this study, we conducted a series of behavioral tests assessing motor performance, emotion and memory in an AD mouse model. In addition we examined the effect of PGRN reduction on amyloid-associated pathology using immunohistochemistry and western blot techniques. We report that PGRN haploinsufficiency in an AD transgenic mouse model results in significant behavioral alterations.

Disclosures: **C. Vollert:** None. **K. Ngo:** None. **H. Pourzargham:** None. **J.L. Eriksen:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.07/D72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: GMU Osher Lifelong Learning Institute Scholarship

Air Force Association Spouse Scholarship

Title: The role of copper deficiency and zinc in a mouse model of early onset Alzheimer's disease

Authors: ***S. N. HOWELL**, P. B. BOZZELLI, J. M. FLINN
Psychology, George Mason Univ., Fairfax, VA

Abstract: While significant advances have been made in understanding the neural mechanisms of Alzheimer's disease (AD), many aspects remain unknown. A more recent theory revolves around the effects of metals in the diseased brain. Building on research previously conducted in our lab, this study examined the roles of two particular metals, zinc (Zn) and copper (Cu), in a mouse model of early onset AD. The aforementioned studies found that excess Zn caused behavioral impairments in rats and mice. Further, it was found that the addition of Cu to the Zn enhanced water remediated the negative effects seen in the purely Zn group; this information supports the theory that excess Zn causes a Cu deficiency. To test this theory it is necessary to look at a Cu deficiency directly. For this purpose, we developed a specialized Cu control and Cu deficient diet (differing only in levels of Cu) with Harlan laboratory nutritionists. Wildtype (Wt)

(C57Bl/6J) and transgenic (Tg) mice with one copy of a mutated human amyloid precursor protein (hAPP) gene (J20, breeders obtained from the Jackson Laboratory) were raised according to one of three groups: a strictly control group (lab water + control diet), or one of two experimental conditions involving excess Zn (Zn water + control diet) and a diet deficient in Cu (+ lab water). Mice were ran in two behavioral tasks, novel object recognition (NOR) and Morris water maze (MWM), aimed at identifying the effects of the metals on memory deficits seen early in AD patients. Mice were tested beginning at 6 months of age. Preliminary data indicates an early protective role of Cu deficiency for the Tg mice. Tg mice on the Cu deficient diet performed better than Tg controls, but not significantly so, and significantly different in some measures of object recognition (NOR) and latency (MWM) than the zinc enhanced animals. Tg Cu deficient mice show similar trends in both tasks to the Wt mice on the Cu control diet.

Disclosures: **S.N. Howell:** None. **P.B. Bozzelli:** None. **J.M. Flinn:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.08/E1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS079637

Title: Modeling the co-morbidity of vascular dementia and amyloid pathology of Alzheimer's disease

Authors: ***E. M. WEEKMAN**, T. L. SUDDUTH, H. M. BROTHERS, K. BRAUN, D. M. WILCOCK
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Abstract: Vascular dementia (VaD) is the second most common cause of dementia behind Alzheimer’s disease (AD) and it is estimated that 40% of AD patients have VaD. Due to a lack of mouse models, VaD is a relatively understudied area and the effects of VaD on AD is also undetermined. The goal of this study was to determine the effects VaD has on amyloid pathology. Induction of hyperhomocysteinemia (HHCy) through a diet deficient in folate, B6, B12 and enriched in methionine in wildtype mice leads to cortical microhemorrhages and cognitive deficits and provides a mouse model to study one form of VaD. In this study, both wildtype (WT) and APP/PS1 transgenic mice aged 6 months were placed on the HHCy or

control diet for 6 months. Cognition was assessed through the radial arm water maze. Aβ levels were quantified using immunohistochemistry, Congo red staining and ELISA measurement. Neuroinflammation was assessed by qPCR for gene markers specific for peripheral macrophage phenotypes. Matrix metalloproteinase (MMP) activation was measured by gelatin zymography and microhemorrhages were assessed by Prussian blue staining. In the radial arm water maze, wildtype mice on the HHCy diet and APP/PS1 mice on control diet were similarly impaired when compared to WT mice on control diet. APP/PS1 mice on the HHCy diet had an even greater impairment than WT mice on the HHCy diet or APP/PS1 mice on control diet. Aβ measurement through both immunohistochemistry and ELISA quantification showed no significant changes, but Congo red staining for dense plaques showed an increase in cerebrovascular amyloid and a decrease in parenchymal amyloid in APP/PS1 mice on the HHCy diet. The HHCy diet induced an M1 phenotype in WT mice and caused a switch from an M2a to an M1 phenotype in APP/PS1 mice. Finally, MMP2 and MMP9 activity and microhemorrhages were increased in WT mice on the HHCy diet and were even higher in APP/PS1 mice on the HHCy diet. Overall, we have successfully modeled mixed dementia through induction of VaD with the HHCy diet and amyloid deposition in APP/PS1 transgenic mice. This mixed dementia results in a neuroinflammatory phenotype switch, increased cerebrovascular amyloid, activation of MMPs and increased microhemorrhages. There is also an additive effect on cognitive outcomes that is similarly seen in human patients with VaD and AD.

Disclosures: E.M. Weekman: None. **T.L. Sudduth:** None. **H.M. Brothers:** None. **K. Braun:** None. **D.M. Wilcock:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

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Program#/Poster#: 406.09/E2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI 20790084

KAKENHI 24590133

Takeda Science Foundation

Smoking Research Foundation

Title: Accelerated impairment of learning and memory by presenilin 2 mutation in APP transgenic mouse model of Alzheimer's disease

Authors: *Y. KIRINO, Y. KISHIMOTO, A. FUKUTA, A. NAGAO, E. HIGASHIHARA
Tokushima Bunri Univ., Tokushima, Japan

Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized clinically by neuropathological features including abnormal deposition of amyloid β (Aβ) peptides, neurofibrillary tangles, and neuronal loss in selective brain regions. Aβ is produced after sequential cleavage of the amyloid precursor protein (APP) by two proteases, β- and γ-secretases, and highly homologous proteins presenilin 1 and 2 (PS1 and PS2) are important components of the γ-secretase complex. In mouse model studies, it was shown that the PS1 or PS2 mutation accelerated AD-like pathologies with increased Aβ42 production through enhanced γ-secretase activity. Furthermore, cognitive deficits in various types of learning tasks have been reported in PS1/APP double transgenic mice. In contrast, however, there was no report of non-spatial hippocampal learning task including contextual fear conditioning or trace eyeblink conditioning in the PS2/APP double transgenic mice. Hence, in the present study, we aimed to investigate whether PS2 mutation accelerates the onset of learning deficits in AD mouse model overexpressing human amyloid precursor protein (APP) with the Swedish mutation (K670N, M671L) (Tg2576 mice). For this purpose, we generated a double transgenic mouse (PS2Tg2576 mice) by cross-breeding transgenic mice carrying human mutant PS2 (N141I) with the Tg2576 mice. We then tested two types of nonspatial memory task, contextual fear conditioning and long-trace interval (trace interval = 500 ms) eyeblink conditioning as well as a spatial memory task, Morris water maze in the PS2Tg2576 double transgenic mice and in Tg2576 mice, at the age of 3, 4, 6, and 12 months. In Tg2576 mice, the onset of learning deficits in fear conditioning and EBCC was observed at 6 months of age while that in MWM was at 10 months. In contrast, in PS2Tg2576 mice the onset of learning deficits in fear conditioning and EBCC was detected at 3 and 4 months of age, respectively, while that was at 6 months in MWM. Our cross-sectional study has clearly indicated that PS2 mutation significantly accelerates the onset of cognitive impairment of nonspatial and spatial task memory and that it is detected in earlier stage in fear and trace eyeblink conditioning than in MWM.

Disclosures: Y. Kirino: None. **Y. Kishimoto:** None. **A. Fukuta:** None. **A. Nagao:** None. **E. Higashihara:** None.

Poster

406. APP and Abeta Pathology Models

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.10/E3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant

Alzheimer's Association and Global Down Syndrome

Jerome's Foundation

Title: Nebula/DSCR1 ameliorates APP-induced learning and memory impairments in *Drosophila*

Authors: *J. SHAW[1,2], K. T. CHANG[1,2]
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Abstract: Despite the inevitability of developing Alzheimer's disease (AD) neuropathologies for most Down Syndrome (DS) individuals, there is a delay in the onset of dementia suggesting the activation of a neuroprotective pathway. AD is characterized by an age-dependent deterioration in the ability to remember newly learned information. Post-mortem brains from DS and AD patients show an upregulation of a gene called DSCR1 (Down syndrome critical region 1 gene) that encodes an inhibitor of calcineurin, but the relationship between DSCR1 upregulation and memory deficits in AD is not well understood. By using the classical Pavlovian olfactory conditioning test, we demonstrate that *Drosophila* with APP overexpression fails to avoid a shock-paired odor in both a learning and memory task. Interestingly, we show that co-upregulation of Nebula, *Drosophila* homolog of DSCR1, protects against APP-induced learning and memory impairments early in life. Here we systematically evaluate how altering different kinase and phosphatase pathways downstream of nebula/DSCR1 influences APP-induced learning and memory deficits during aging. A greater understanding of the cell signaling pathways abnormally regulated in aging Alzheimer's and Down syndrome patients has the potential to identify targets for therapeutic intervention in delaying declines in learning and memory performance.

Disclosures: J. Shaw: None. **K.T. Chang:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 3T32NS043124-12S1

Title: Early amyloid deposition in the anterior olfactory nucleus correlates with specific mixture discrimination deficits in 5xFAD mice

Authors: *D. K. MURPHEY[1], D. KOYYAGUNTALA[2], B. ARENKIEL[1]
[1]Neuroscience, Genet., Baylor Col. of Medicine/Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [2]Duke Univ., Durham, NC

Abstract: The same circuits that subserve complex odorant processing are exquisitely susceptible to dysregulation/attrition in neurodegenerative conditions such as Alzheimer's Disease (AD). Studies reveal that early olfactory phenotypes coincide with the onset of memory impairment. In both humans and mouse models of AD, Aβ plaque deposition occurs early in the anterior olfactory nucleus (AON). The 5xFAD mouse represents a robust AD model in its early and severe amyloid pathology, memory deficits, and pyramidal cell loss. However, olfactory deficits in this model have not been well characterized either anatomically or functionally, an endeavor which may prove diagnostically useful in AD patients, who demonstrate early compromise of olfaction. To determine the earliest olfactory area affected by AD, we performed immunohistochemical stains of Aβ in mice ages 4 months, 6 months, and 12 months and found that marked plaque deposition is present in the AON prior to the main olfactory bulb (MOB). Unlike the MOB, which processes mostly monomolecular odorants, the AON is thought to process odorant mixtures. We therefore devised a behavioral screen in which 8 2-4 month old 5xFAD animals and 6 age-matched controls performed a cross-habituation task using monomolecular pairs and mixtures of varying degrees of constituent monomolecular odorant overlap. We found that while the control animals could perform all discriminations ($p < 10\text{-}4$ for monomolecular and mixtures of intermediate overlap, $p < 0.02$ for mixtures with more overlap), 5xFAD animals could only perform discriminations of monomolecular pairs ($p < 0.04$). Determining the anatomical and functional abnormalities in an experimentally tractable system affected early in AD will provide opportunities to identify early disease diagnostic markers.

Disclosures: D.K. Murphey: None. **B. Arenkiel:** None. **D. Koyyaguntala:** None.

Poster

406. APP and Abeta Pathology Models

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Program#/Poster#: 406.12/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA 1R15AG039008

Title: Modulatory effects of prostacyclin in the mouse model of Alzheimer's dsease

Authors: *J. ERIKSEN[1], C. VOLLERT[2], M. SCHMITT[2], O. OHIA[2], S. MONTAZARI[2]
[1]Pharmacol. and Pharmaceut. Sci., [2]PPS, Univ. of Houston, Houston, TX

Abstract: Alzheimer's disease associated with development of both amyloid and tau pathologies, and alterations in the neurovascular compartment, that have been hypothesized to play a role in the progression of pathology and cognitive decline. Three decades ago, prostacyclin, an extremely short-lived endogenous lipid metabolite, was first used for the treatment of pulmonary hypertension; this prostanoid has potent vasodilatory, anti-atherothrombotic and pro-angiogenic effects, and is suggested to be protective to neurovascular function, but little is known about its role within the CNS. We hypothesized that elevated prostacyclin expression might be protective in Alzheimer's disease. In this study, we have characterized the effect of constitutively elevated prostacyclin biosynthesis in an APP-overexpressing mouse model of Alzheimer's disease. We found that elevated prostacyclin levels exerted significant effects on learning and behavior, and induced significant changes in pathology. Our data suggest that prostacyclin may be a potential therapeutic target for amyloid-associated pathologies within the CNS.

Disclosures: J. Eriksen: None. **C. Vollert:** None. **M. Schmitt:** None. **O. Ohia:** None. **S. Montazari:** None.

Poster

406. APP and Abeta Pathology Models

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Program#/Poster#: 406.13/E6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFG: SFB779/B6

CBBS 1211080005

Title: Cued and contextual fear learning in APP/PS1 mice

Authors: *T. ENDRES[1], G. HÖLZL[1], L. PSOTTA[1], E. EDELMANN[1], V. LESSMANN[1,2]
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Abstract: To identify novel treatment approaches for Alzheimer's disease (AD) is probably one of the most challenging topics in neuroscience. A couple of studies demonstrated that emotional processing like the recognition of fearful faces or the learning of fear is impaired in AD patients. Interestingly, these impairments occur already at early stages of the etiopathology of AD. Thus, altered emotional processing might be regarded as an early symptom in the development of AD. In the present study, we analyzed different aspects of fear learning in differently aged APP/PS1 mice. This mouse model for AD combines the Swedish APP (KM670/671NL) mutation with the PS1-L166P mutation under control of the Thy1 promoter (Radde et al., 2006, EMBO), resulting in a constant post-developmental expression of Aß and subsequent plaque formation. By testing amygdala-dependent cued fear learning, we observed only slight impairments in 12 months old APP/PS1 mice. In the adjacent fear extinction training, we observed no impairments in the extinction of these cued fear memories, neither in short nor in long-term extinction memory. In contrast to the cued fear learning, we observed deficits in contextual fear learning in six months old APP/PS1 animals. However, the subsequent extinction of these contextual fear memories remained intact. As a non-emotional control experiment we also tested the object recognition memory of these animals and observed no impairments in the short-term memory of these animals. Currently, we are analyzing the amounts of Aß40/42 proteins in the hippocampus, amygdala and medio-prefrontal cortex of the tested animals in order to correlate the local occurrence of these toxic Aß-species with the behavioral performance of the animals. In addition, we started to analyze long-term potentiation (LTP) in hippocampal slices of these mice. Here, first results indicate an impaired LTP in the CA1 region of six months old APP/PS1 mice. In conclusion, these results demonstrate a selective impairment in contextual fear learning in middle-aged APP/PS1 mice. Ongoing experiments aim at detecting a possible inter-individual correlation between expression levels of soluble forms of Aß protein and altered hippocampal synaptic plasticity. This work was supported by the Center for behavioral brain sciences (CBBS) and the Deutsche Forschungsgemeinschaft (DFG, SFB 779/B6).

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Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.14/E7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fondation Lejeune and LECMA grant n°09710

Title: Age and human Aβ-dependent effect of PS1 mutation in development of Alzheimer-like pathology: Studies in transgenic knock-in mice for Alzheimer's disease mutant Presenilin-1 (PS1(M146V)) and wild-type human APP (hAPPwt)

Authors: *H. S. ZANJANI[1], K. KINUGAWA[1,2], M. DOULAZMI[1,2], M. P. MATTSON[3], J. MARIANI[1,2], C. ROVIRA[1,2]
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Abstract: Memory impairment is the major and early cognitive symptom of Alzheimer disease (AD) and has been linked to synaptic deficits. Understanding the mechanisms of these synaptic deficits is essential for preventing dementia. The vast majority of familial AD (FAD) cases are related to presenilin-1 (PS1) mutation. PS1 is part of the gamma-secretase complex and involved in the release of the neurotoxin amyloid Aβ, and PS1 variants activate GSK3β and cause Tau hyperphosphorylation. There are evidences that, independently of its effect on amyloid secretion, PS1 influences the synaptic function and transmitters release. In PS1(M146V)KI transgenic mice there is an overproduction of Aβ42, but murine Aβ fails to aggregate and it is much less toxic than human Aβ. In this study we crossed PS1(M146V)KI and overexpressing wild type human APP AD mice models (hAPPwt) to generate double transgenic hAPPwt/PS1(M146V)KI mice that can secrete human Aβ. In these mice we tested the effect of PS1 mutation on the neuronal function and synaptic plasticity in the presence of human Aβ. We recorded in the CA1 region from hippocampal slices the early LTP in 9-month-old mice. We found no significant differences between control mice: +/+ mice (1,29 ± 0,0027, n=11); +/APP mice (1,242 ± 0,0027, n=10); PS1 KI/KI mice (1,2563 ± 0,0032, n=10). On the other hand there was a clear deficit in the early LTP in the hAPPwt/PS1(M146V)KI mice (0,0997 ± 0,0018, n=11). Therefore this study revealed early Aβ-dependent synaptic deficits caused by the PS1(M146V)KI mutation. For imunohistopathological analysis, brain sections from young and aged WT and transgenic mice were immunostained with anti- β-amyloid protein and anti-paired helical filament-tau (PHF-tau) antibodies. Intracellular immunoreactivities for PHF-tau staining were observed in the transgenic but not in the wild-type mice brains. We found a deposition of fibrillar β-amyloid within cerebral vessels, indicating presence of a cerebral amyloid angiopathy (CAA), in the PS1(M146V)KI, hAPPwt and double transgenic mice at the age of 15 and 21 months but not in the WT mice. An amyloid plaque deposition was observed as well in the 21-month-old double transgenic mice brain indicating progression of PS1 induced pathology in presence of human Aβ and aging.

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Poster

406. APP and Abeta Pathology Models

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Program#/Poster#: 406.15/E8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1 R21AG043813-01A1

Title: Voluntary exercise leads to preservation of spatial memory in the TgSwDI mouse model of cerebral microvascular amyloid without reducing Aβ

Authors: M. E. ANDERSON[1], M.-H. OU-YANG[2], F. XU[2], A. KUMAR[1], G. SINGH[1], T. SHUB[1], B. J. ANDERSON[1], W. E. VAN NOSTRAND[2], *J. K. ROBINSON[3,1]
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Abstract: Despite recent advances in our understanding of the mechanisms that underlie Alzheimer's disease (AD) and related disorders, current pharmacological treatments are only partially effective in improving symptoms. In light of this, modification of life-style factors has become increasingly attractive as a means of delaying the onset or slowing the progression of AD. Epidemiological studies have shown cardiovascular exercise to protect against cognitive impairment in AD. Studies in murine models of AD also suggest that cardiovascular exercise improves cognitive function and some report corresponding alterations of AD pathology. Presently, we investigate the specific effects of voluntary cardiovascular exercise on vascular amyloidosis by using the Tg-SwDI transgenic mouse model of cerebral amyloid angiopathy. Tg-SwDI mice express low levels of familial Dutch/Iowa CAA mutant human APP and develop fibrillar Aβ exclusively in the cerebral microvasculature in the thalamus, subiculum and forebrain, beginning at 3-4 months. Age-matched female Tg-SwDI mice were given 24-hour access to exercise wheels in their home cage for 4 months. At 7.5 months of age, spatial memory and exploratory behavior were assessed in the Barnes maze and 8 radial arm maze. Brain tissue was harvested for quantitative ELISA, as well as immunostaining, for Aβ species. Our results indicate that 4 months of voluntary exercise leads to a preservation of spatial memory task performance without decreasing pools of Aβ or amyloid deposition in the cerebral vasculature and suggest that long-term voluntary exercise provides benefits to cognitive function through targets downstream of Aβ deposition.

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Poster

406. APP and Abeta Pathology Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MNIRGD-12-243075

Title: titration of biologically active amyloid-β seeds in a transgenic mice model of alzheimer's disease

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Abstract: A hallmark feature of Alzheimer's disease (AD) is the misfolding, aggregation and brain accumulation of amyloid-β (Aβ) and tau proteins. AD is considered as a Protein Misfolding Disorder (PMDs), a group of diseases characterized by the misfolding of a native protein into a toxic isoform. PMDs comprise several pathological conditions, including Parkinson's disease, and Transmissible Spongiform Encephalopathies (TSEs) or prion diseases. TSEs are so far the only PMDs considered as infectious. Recent evidence suggests that misfolding of disease-associated Aβ can be transmitted following a prion-like mechanism. However, additional studies need to be done in order to analyze whether Aβ has the biological properties of prions. One of these characteristics is that the extent of pathological transmission should directly depend on the amount of Aβ aggregated seeds administered, i.e. the material must be titrable in transmission bioassays. The aim of this work was to titrate Aβ aggregates in an animal model of AD (tg2576), as well as to find the minimum amount of particles able to accelerate amyloid pathology *in vivo*. A pool of brain extracts coming from old Tg2576 animals was serially diluted. Samples from each dilution were injected into the hippocampus of young tg2576 mice (50 days old). Animals were sacrificed at 285 days old and brain samples were analyzed for amyloid pathology by IHC and ELISA. As previously shown, administration of misfolded Aβ containing brain materials was able to seed the aggregation of endogenous Aβ in Tg2576 mice. A significant increase was

observed for animals injected with the brain dilutions ranging from 10^{-1} to 10^{-6}. Animals injected with the sample representing the higher dilution (10^{-7}) did not show any significant difference when compared to non-injected controls. Importantly, the burden of aggregates observed in animals injected with the samples bearing the highest concentration of Aβ seeds was increased ~400 folds. The titration curve obtained in this experiment was compared to the natural Aβ load spontaneously accumulated by these mice overtime. Our findings suggest that administration of the largest dose of Aβ seeds led to an acceleration of pathology equivalent to several months. Our findings, together with previously published reports, suggest that some aspects of AD pathology might be transmissible. These results show that active Aβ seeds present in the brain of old Tg2576 mice can seed brain amyloidosis in a titrable manner, similarly as observed for infectious prions. These results may contribute to understand the mechanisms implicated in the initiation of Aβ pathology and therefore be useful to develop new therapeutic strategies.

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Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.17/E10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Antidepressant drug restores expression levels of M-type potassium channels (KCNQ2) in Alzheimer's disease animal model (Thy1-APP)

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to dementia and death. Despite the intense efforts studying AD, we still do not understand its cause(s), and this adversely affects our ability to develop effective treatments and means to prevent it. Recent reports have shown that the antidepressant drug, fluoxetine (Flx), enhances memory and cognition in AD patients. We decided to investigate the influence of fluoxetine over the expression of the potassium channel KCNQ2 in the hippocampus using the McGill Thy1-APP transgenic model carrying the Indiana and Swedish double mutations in the human APP,

which leads to an overproduction of amyloid β peptides (Aβ) and cognitive dysfunction. Previous results show that at the age of 7 months, LTP is affected in the model perhaps due to the distinct presence of plaque formation. Groups were treated for 21 days with saline (placebo) or Flx by intraperitoneal injections in doses of 5mg/kg (diluted in 0.9% saline, total volume will be 100µl). The placebo group included wild-type (n=3) and transgenic mice (n=3) as controls. The Flx treated group included wild-type (n=4) and transgenic mice (n=6). Our results show that KCNQ2 protein levels in wild-type mice, both placebo vs Flx treated groups did not differ. When we compare the placebo groups, lower levels of KCNQ2 where found in the transgenic group vs the wild-type (p=0.01). Another significant difference was observed between transgenic mice, were the treated with Flx showed increased levels of the protein vs the mice that only receive saline solution (p=0.01). When the wild-type and transgenic groups receive Flx, the levels of KCNQ2 did not differ among them, proving that the use of the antidepressant can restore the protein values to the levels observed in animals without APP deposition or AB plaque formation. The information provided showed that fluoxetine treatment can restore KCNQ2 to normal values in treatment for AD.

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Poster

406. APP and Abeta Pathology Models

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Program#/Poster#: 406.18/E11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC

Title: Age-related changes in feeding behaviour, activity levels, hormones and metabolism in female 5xFAD mice

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Abstract: The 5xFAD mouse is a double transgenic model of Alzheimer's disease (AD) which carries an amyloid precursor protein (APP) transgene with three mutations and a presenilin-1 transgene with two mutations. The 5xFAD mice have increased beta amyloid (AB)-peptides and develop AB-plaques by two months of age. Weight-loss is a common problem in human AD

patients and we have observed age-related weight-loss in 5xFAD mice. We therefore investigated age-related changes in activity levels, feeding behaviour, body weight, body composition, and feeding-related hormones in female 5xFAD mice and their WT (C57BL/6JxSJL/J F1) controls from 3 to 12 months of age. Levels of grooming and rearing did not differ but 5xFAD mice showed less climbing and jumping ($p<0.05$) and spent more time remaining still ($p < 0.05$) than WT mice. No differences were found in food intake between genotypes but WT mice weighed more than 5xFAD mice ($p < 0.05$). Muscle tissue was collected to evaluate whether mice suffer from sarcopenia (muscle loss) but 5xFAD mice did not differ in muscle mass compared to WT mice. However, the 5xFAD mice had less fat than WT mice ($p < 0.05$). There were no differences in plasma insulin levels or glucose concentrations between genotypes. We conclude that the 5xFAD mice are hypoactive compared to WT mice, that the 5xFAD mouse model does not suffer from sarcopenia, and that the 5xFAD mice have less fat than WT mice, suggesting fat loss is the main contributor to weight-loss in this mouse model. We found no abnormalities in blood glucose or insulin levels in this mouse model, and the poster will report on plasma concentrations of ghrelin and leptin.

Disclosures: W.H. Gendron: None. **S. Pelletier:** None. **R.E. Brown:** None. **Y. Anini:** None.

Poster

406. APP and Abeta Pathology Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG027224

VAPHS BX000452

Title: A mouse model with distinct features of the Alzheimer disease with psychosis phenotype

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Abstract: Psychosis in Alzheimer's disease denotes a more severely progressive phenotypic variant of the disease, characterized by more rapid decline in cognition compared to AD without psychosis (AD-P). AD+P aggregates within families with an estimated heritability of 61%. In dorsolateral prefrontal cortex of AD+P cases, we previously found increased disruption of synapse integrity, lower kalirin expression, and greater Aβ42/Aβ40 ratio. As kalirin signaling is particularly important to dendritic spine function in the cortex, lower kalirin expression in AD+P may contribute to the cognitive profile of the AD+P phenotype. To assess the relative contributions of reduced kalirin expression and β-amyloid overproduction *in vivo*, we have generated a genetic mouse model aiming to recapitulate the distinct cognitive and neuropathologic deficits of AD+P. We paired double transgenic Borchelt mice obtained from the Mutant Mouse Regional Resource Center (B6C3- Tg(APPswe,PSEN1dE9) 85Dbo/Mmjax, Jax #004462, [APPswePS1]) with kalirin heterozygotes (Kal+/-) rederived on a C57Bl/6J background. All four genotypes resulting from this cross (Kal+/+APPswePS1 mice, Kal+/-APPswePS1, Kal+/-, WT) were viable and represented in the approximate expected Mendelian ratios. Both Kal+/+APPswePS1 mice and Kal+/-APPswePS1had significantly greater mortality postweaning than WT. Male mice resulting from this cross (n = 11-16/genotype) were evaluated at six months of age in open field (OF), spontaneous alternation (SA), acoustic startle (AS) and prepulse inhibition (PPI) of AS tests. After testing, mice were sacrificed and their cerebral cortex isolated and frozen at -80°C for targeted proteomic analysis. Behavioral performance of both Kal+/+APPswePS1 mice and Kal+/-APPswePS1 was impaired compared to WT on a summary measure of all tests. On individual tests, Kal+/-APPswePS1 mice exhibited significantly greater activity in the OF test compared to WT, as did Kal+/+APPswePS1. Conclusion: At six months of age, when Kal+/+APPswePS1 are just beginning to show significant Aβ deposition, reduced levels of kalirin protein did not appreciably alter behavioral deficits associated with incipient neuropathological features. Future studies will use targeted proteomics to quantify synaptic protein alterations in these animals, and will assess the combined impact of reduced kalirin levels and β-amyloid overproduction on behavior and the synaptic proteome in older animals with extensive amyloid deposition.

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Poster

406. APP and Abeta Pathology Models

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DA034681

Title: Genome-wide transcription and DNA methylation profiling in an APP mouse model of Alzheimer's disease

Authors: *M. C. GUZMAN-KARLSSON[1], Z. LI[1], J. J. DAY[1], E. D. ROBERSON[2], J. D. SWEATT[1]
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Abstract: Epigenetic mechanisms are regulated in response to experience, allowing for dynamic, bidirectional regulation of gene expression necessary for neuronal plasticity and long-term behavioral memory. Evidence from our lab and others suggests that dysregulated epigenetic mechanisms, like post-translational modifications of histones and DNA methylation, may contribute to the neuronal dysfunction and cognitive impairment in Alzheimer's disease (AD). Previous research has focused on the role of histone acetylation with observed reductions in global and locus-specific histone acetylation in animal models of AD that are associated with repressed transcription of memory-related genes. Furthermore, pharmacological inhibition of histone deacetylase (HDAC) enzymes, a manipulation that effectively increases histone acetylation, ameliorates deficits in gene expression, synaptic plasticity and hippocampus-dependent memory. Recently, attention has turned towards DNA methylation, a similarly potent regulator of transcription, plasticity, and behavior that shares extensive cross-talk with histone modifications. Although emerging data suggest that the AD brain might be characterized by global hypomethylation as well as locus-specific hypermethylation, a comprehensive understanding of the role of DNA methylation in AD is still lacking. To further examine this topic we utilized the hAPPJ20 AD mouse model in combination with next-generation sequencing technologies to systematically characterize genome-wide alterations in hippocampal gene expression and DNA methylation. Identification of biologically relevant loci with altered DNA methylation will further our understanding of the role of DNA methylation in amyloid-beta (Aβ)-induced transcriptional dysregulation and cognitive impairment.

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Poster

406. APP and Abeta Pathology Models

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The Research Council of Norway

EC Human Brain Project

Title: Transgene expression in the neuropsin tTA driver line is not restricted to the entorhinal cortex

Authors: T. B. LEERGAARD[1], M. J. YETMAN[2], S. LILLEHAUG[1], J. G. BJAALIE[1], *J. L. JANKOWSKY[2]
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Abstract: The entorhinal cortex (EC) plays a central role in episodic learning and memory formation, and is among the earliest sites of neuronal loss and neurofibrillary tangle formation in Alzheimer's disease. The EC has therefore been an attractive target for genetic manipulation to selectively modify gene expression or neuronal function in various models of neurological disease. Many such conditional models utilize the neuropsin (Nop) promoter to limit spatial distribution of the tetracycline transactivator (tTA). When crossed with a second tet-responive transgenic line, the resulting bigenic mice will express the transgene of interest in neurons where tTA is active. The Nop-tTA mouse line was reported to restrict tet-responsive transgenes to the superficial layers of medial EC and parts of the pre- and parasubiculum (Yasuda and Mayford, Neuron, 2006), and Nop-tTA mice have been used in several experimental studies examining functional properties of the entorhinal-hippocampal circuitry. The utility of this transgenic driver line is contingent on the specificity of the spatially restricted gene expression, yet detailed neuroanatomical mapping of its expression has not yet been done. We therefore crossed the Nop-tTA driver line with a reporter strain expressing β-galactosidase, and established an online

histological atlas of Nop-tTA regulated gene expression. This atlas resource (available through the Rodent Brain Workbench, www.rbwb.org) was used to perform a detailed brainwide analysis of β-galactosidase labeling in bigenic (Nop-tTA-LacZ) mice. Our findings highlight strong expression in regions beyond the EC and suggest caution in interpreting experiments that depend on precise localization of gene products controlled by the Nop-tTA driver.

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Poster

406. APP and Abeta Pathology Models

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Program#/Poster#: 406.22/E15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 5R01AG041135

Title: A novel target protects against APP-induced age-related neurodegeneration

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Abstract: Alzheimer's disease (AD) is the sixth leading cause of death in the US, affecting more than 5 million people with sadly no treatment in sight. AD is characterized by protein aggregates and selective degeneration of cholinergic neurons. The sigma receptor family and progesterone receptor membrane component 1 (PGRMC1) have been implicated as mediators of cell death, though a specific role for the sigma receptor 2 (Sig2R) and PGRMC1 in neurodegeneration has not been shown. It is hotly contested whether Sig2R and PGRMC1 is the same target; thus here we use Sig2R as a pharmacological designation and PGRMC1 for the gene. To test for a neurodegenerative role of Sig2R/PGRMC1, we used a *C. elegans* model of neurodegeneration that expresses a single extra copy of the human Amyloid Precursor Protein (*APP*) gene. This SC_APP model displays progressive, age-dependent changes in APP protein regulation in specific cholinergic neurons that subsequently die. Moreover, PGRMC1 has a highly conserved sequence and function in this animal. We found that PGRMC1 null alleles significantly reduced the selective degeneration of cholinergic neurons in older adult SC_APP animals but had little effect on baseline neurodegeneration in control animals. We have previously determined that the accumulation of APP protein in our SC_APP model precedes the apoptosis of cholinergic

neurons. To ascertain if PGRMC1 plays a role upstream of abnormal protein accumulation, we asked if deletion of PGRMC1 in our SC_APP model altered patterned accumulation of APP in the neurons susceptible to degeneration. SC_APP animals bearing a deletion of *PGRMC1* showed significantly lower levels of accumulated APP compared to age-matched middle-aged SC_APP adults with a WT copy of PGRMC1. These data indicate that PGRMC1 can be targeted to restrict the inappropriate accumulation of APP that in turn leads to neuron death in our SC_APP model. To find molecules that might influence the PGRMC1 pathway we screened novel Sig2R ligands for the ability to reduce neurodegeneration. We found two compounds that increase neurodegeneration in both the SC_APP model and control strains. More importantly, we found two compounds that decrease APP accumulation and neurodegeneration in the SC_APP model. These Sig2R ligands do not further reduce neurodegeneration in the presence of PGRMC1-directed knockdown suggesting that Sig2R and PGRMC1 act in the same pathway. Together, our results indicate that Sig2R/PGRMC1 can be targeted to reduce both protein accumulation and neurodegeneration providing promising target(s) for age-related neurodegenerative diseases.

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Poster

406. APP and Abeta Pathology Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG017173 (to RPF)

Title: Nitric oxide, platelet and microvascular involvement in AD pathophysiology in aging APP transgenic mice

Authors: R. JAGADAPILLAI[1], A. M. ROBERTS[2], R. VAISHNAV[3], R. P. FRIEDLAND[4], L. R. SACHLEBEN, Jr.[1], *E. GOZAL[5]
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Abstract: Alzheimer’s disease (AD) is associated with vascular pathology, small vessel disease, oxidative stress, inflammation and deficiencies of the blood brain barrier (BBB). Vascular injury

is associated with Aβ formation and compromises its clearance, contributing to the pathological evolution of Alzheimer's disease. We have previously shown increased superoxide production, lipid peroxidation, and gliosis in a transgenic mouse model of AD, expressing the human APP gene 770 isoform, with the Swedish, Dutch and Iowa mutations in neurons, under the control of the mouse Thy1.2 promoter. This genetic alteration causes amyloid deposits with Aβ plaque accumulation in parenchyma and cerebral vessels, apparent at 6 months and fully developed at 12 months. Fibrinogen accumulation, interacting with Aβ may also affect clot formation and degradation. NO regulates platelet function and affects fibrinogen binding to platelets and the endothelium, suggesting a central role for platelets in AD physiopathology. Therefore, we investigated a potential role for microvascular injury and platelets in the pathology developed by TgAD mice. We assessed the expression of inducible nitric oxide (iNOS), 3-nitrotyrosine (3-NT), fibrinogen and CD61, a marker of platelets and megakaryocytes in 12 month old wild type mice (WT) and transgenic AD mice (TgAD). Immunohistochemical staining showed significant increases in iNOS, 3-NT, fibrinogen and CD61 cortical expression in TgAD mice compared to WT. While fibrinogen staining appeared localized in WT, the staining seemed more diffuse and abundant in TgAD mice. Increased iNOS expression and superoxide formation in TgAD mice result in enhanced peroxinitrite expression and protein nitrosylation, decreasing NO bioavailability, thus affecting platelet function. These data indicate that Aβ accumulation in TgAD mice is associated with increased fibrinogen and platelet adhesion which in turn may enhance Aβ plaque accumulation.

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Poster

406. APP and Abeta Pathology Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant NS085171

Title: Epigenetic gene regulation in alzheimer's disease: Neuroprotection versus neuroplasticity

Authors: *J. YOU, B. F. CORBETT, X. ZHANG, A. HAZRA, J. CHIN
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Abstract: Alzheimer's disease (AD) is characterized by progressive cognitive decline and severe memory loss. As current therapies for AD offer limited benefit, it is critical to better understand the mechanisms underlying cognitive decline in AD. The key finding that AD is associated with seizures may provide valuable insight into these mechanisms. Recent evidence suggests that seizures in AD are not just incidental, but may rather play an active role in contributing to cognitive decline early in disease progression. Consistent with this, treatment of both AD patients and AD mouse models with antiepileptics improves cognitive function. Therefore, understanding the mechanisms by which seizures contribute to cognitive impairment so may enable the discovery of novel strategies to improve cognitive function. Our studies of seizure-related changes in the hippocampus of a transgenic AD mouse model, as well as a pharmacological model of epilepsy, have revealed that expression of the transcription factor ΔFosB is markedly increased by seizures. ΔFosB has an unusually long half-life (on the order of weeks) and interacts with various histone modification enzymes. Identification of key ΔFosB gene targets in AD mice suggests that ΔFosB may serve a neuroprotective function during states of chronic neuronal excitation (such as in epilepsy and AD), but may do so at the cost of synaptic plasticity. Thus, delineation of the actions of ΔFosB in the hippocampus may provide critical insight into the mechanisms by which seizures induce synaptic dysfunction and cognitive impairment in neurological conditions such as epilepsy as AD.

Disclosures: J. You: None. **B.F. Corbett:** None. **X. Zhang:** None. **A. Hazra:** None. **J. Chin:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.25/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Leon Levy Neuroscience Fellowship

NIH Grant NS37853

Title: Amyloid-beta (Aβ)-induced depolarization and abnormal responses to leptin in hypothalamic neuropeptide Y (NPY) neurons

Authors: *G. WANG, M. ISHII, C. IADECOLA
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Abstract: Leptin is an adipocyte-derived hormone that negatively regulates feeding and energy homeostasis, mainly through hypothalamic pathways. In a companion presentation (Ishii et al., this meeting), we expand on our prior studies demonstrating that mice overexpressing the Swedish mutation of amyloid precursor protein (Tg2576) exhibit: (a) low fat mass and pathologically low plasma leptin levels, prior to cognitive decline and amyloid plaques, and (b) lack of the expected neuropeptide Y (NPY) transcriptional response to the low leptin state in the hypothalamus (Ishii et al., SFN 2013). Here, we examined whether the electrophysiological characteristics of hypothalamic NPY neurons are altered in Tg2576 mice and if exogenous $A\beta_{1-42}$ could recapitulate such alterations. Tg2576 mice expressing GFP in NPY neurons were developed by crossing male Tg2576 mice with female NPY-GFP mice and studied at 2-5 weeks of age. Using whole-cell current-clamp in hypothalamic slices (total 69 cells), we found that Tg2576 NPY neurons of the arcuate nucleus have a less negative resting potential (-63.1±4.5 mV; mean±SE) and a reduced spontaneous activity (2.2±0.3 Hz) than WT mice (-73.2±2.0 mV; $p<0.05$, n=10-17 cells/group; WT: 8.5±0.1 Hz; $p<0.01$). Application of leptin (100 nM) hyperpolarized NPY neurons in WT (-9±1.8 mV; $p<0.01$), but not in Tg2576 slices (-1.7±1.8 mV; $p>0.05$). Leptin did not affect spontaneous activity both in WT ($p>0.05$) and Tg2576 NPY neurons ($p>0.05$). On the other hand, application of ghrelin (100 nM), a peptide with physiological effects opposite to leptin (Neuron, 37:649), depolarized NPY neurons in WT slices (+19.9±5 mV; $p<0.01$), an effect attenuated in Tg2576 slices (+5.7±2.1 mV; $p>0.05$). Ghrelin did not affect spontaneous activity in NPY neurons both in WT ($p>0.05$) and Tg2576 slices ($p>0.05$). Oligomeric $A\beta_{1-42}$ (3-100 nM) depolarized WT NPY neurons dose-dependently (EC_{50}=29.7 nM). Furthermore, $A\beta_{1-42}$ (100 nM) suppressed leptin-mediated hyperpolarization (+0.6±0.5 mV, $p>0.05$ form control), and inhibited the depolarizing response to ghrelin (3-100 nM; IC_{50}=10.2 nM). Spontaneous activity was not affected by $A\beta_{1-42}$ ($p>0.05$). These data indicate that oligomeric $A\beta_{1-42}$ disrupts hypothalamic responses to leptin and ghrelin by changing the excitability of arcuate NPY neurons, recapitulating the electrophysiological alterations observed in Tg2576 mice. Collectively, the findings identify hypothalamic NPY neurons as a previously unrecognized target of the deleterious effects of $A\beta_{1-42}$, which may underlie the body weight deficits and pathologically low leptin state seen in young Tg2576 mice and, possibly, in patients with early-stage Alzheimer's disease.

Disclosures: G. Wang: None. **M. Ishii:** None. **C. Iadecola:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

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Program#/Poster#: 406.26/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Leon Levy Neuroscience Fellowship

NIH Grant NS37853

Title: Hypothalamic leptin signaling deficits underlie the metabolic dysfunction in mice overexpressing amyloid precursor protein

Authors: *M. ISHII, G. WANG, C. IADECOLA
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Abstract: Weight loss is a prominent feature of Alzheimer's disease (AD) that often occurs before the mental decline and worsens as AD progresses (Arch Neuro 63: 1312, 2006). Low plasma levels of leptin, a fat derived hormone that acts on the hypothalamus to regulate body weight, are associated with increased risk of AD (JAMA 302: 2565, 2009). We previously found that mice overexpressing mutated forms of the amyloid precursor protein (Tg2576) have lower body weight, adiposity and plasma leptin compared to wild-type (WT) littermates, prior to amyloid plaque formation or cognitive decline (Ishii et al., SFN 2013). In addition, the transcriptional response of hypothalamic NPY to the low leptin state was abnormal. We tested the hypothesis that hypothalamic leptin signaling is altered in Tg2576 mice, leading to the low leptin state and associated metabolic dysfunction. To this end, we first used phospho-STAT3 immunohistochemistry to evaluate the hypothalamic signaling response to alterations in circulating leptin in Tg2576 and WT littermates. Acute leptin administration (5mg/kg i.p., n=4/group) led to robust increases in phospho-STAT3 throughout the hypothalamus. However, compared to WT mice, Tg2576 mice had a reduced number of phospho-STAT3 positive cells in the arcuate nucleus (-22±3.4%; $p<0.05$), a critical target of leptin. In a companion presentation (Wang et al. this meeting), we show that the excitability of arcuate NPY neurons is altered by exogenous amyloid-beta1-42 and in Tg2576 mice, providing the electrophysiological correlate of the hypothalamic dysfunction. Next, we evaluated the effects of aging and increasing brain amyloid burden on the metabolic deficits in 14 month-old Tg2576 mice. Aging enhanced the reduction in body weight (3 months: -12±1.3%, 14 months: -24.9±1.2%, n>6/group, $p<0.01$) and plasma leptin levels (3 months: -47.5±7.1%, 14 months: -86.6±3.2%, n=4-15/group, $p<0.05$) in Tg2576 mice. The effect was associated with reduced food intake (-15.6±2.3%, n=6/group, $p<0.05$) and lower plasma insulin levels (-32.6±4.7%, n>4/group, $p<0.05$), changes not seen in younger mice. This suggests that with aging and advanced amyloid pathology alterations in feeding behavior contribute to the metabolic deficits. We conclude that leptin dysfunction is a critical factor in the metabolic alterations observed in Tg2576 mice prior to amyloid deposition and cognitive deficits. The data establish that leptin signaling in hypothalamic neurons is highly

vulnerable to the deleterious effects of amyloid-beta leading to metabolic dysfunction, and, as such, represents a potential therapeutic target for AD. Supported by Leon Levy Fellowship (M.I.) and NS37853 (C.I.).

Disclosures: **M. Ishii:** None. **G. Wang:** None. **C. Iadecola:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.27/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG026330

NIH 1R01AG045819

Title: Behavioral and histopathological analysis of Ames dwarf x Alzheimer's disease (APP/PS1) mice

Authors: ***K. L. PUIG**, S. G. RAKOCZY, H. M. BROWN-BORG, C. K. COMBS
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Abstract: APP/PS1 double transgenic mice expressing human mutant amyloid precursor protein (APP) and presenilin-1 (PS-1) demonstrate robust brain Aβ peptide containing plaque deposition, increased markers of oxidative stress, behavioral dysfunction, and proinflammatory gliosis. On the other hand, lack of growth hormone, prolactin, and thyroid-stimulating hormone due to a recessive mutation in the Prop 1 gene (*Prop1df*) in Ames dwarf mice results in a phenotype characterized by potentiated anti-oxidant mechanisms, improved learning and memory, and increased longevity in homozygous mice. Based upon this, we hypothesized that a similar hormone deficiency might attenuate disease changes in the brains of APP/PS1 mice. To test this hypothesis, APP/PS1 mice were crossed to the Ames dwarf mouse line and brains and behavior were examined. APP/PS1 x Df/Df, APP/PS1 x Df/+, APP/PS1, wild type, Df/+, and Df/Df mice were compared at 6 months of age by behaviorally testing and assessing amyloid burden, reactive gliosis, and brain cytokine levels. As expected, Df/Df mice demonstrated attenuated brain growth hormone (GH) and insulin-like growth factor 1 (IGF-1) concentrations. This correlated with attenuated astrogliosis and microgliosis in the APP/PS1 x Df/Df mice and, surprisingly, attenuated Aβ plaque deposition and Aβ 1-40 and Aβ 1-42 concentrations.

APP/PS1 x Df/Df mice also demonstrated significantly elevated brain levels of multiple cytokines in spite of the attenuated gliosis. Finally, the APP/PS1 x Df/Df mice also demonstrated improved behavioral performance. These data suggest that by crossing these two lines we created a unique resource in which to study aging and resistance to disease.

Disclosures: K.L. Puig: None. **S.G. Rakoczy:** None. **H.M. Brown-Borg:** None. **C.K. Combs:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.28/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG045819

Title: Amyloid precursor protein localizes in pancreatic islets

Authors: *J. KULAS, C. K. COMBS
Basic Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Alzheimer's disease (AD) is a neurodegenerative condition characterized by brain accumulation of amyloid beta peptide containing plaques and hyperphosphorylated tau containing tangles. A growing body of literature has also demonstrated a possible link between AD and dysfunction in insulin signaling, including an increased prevalence of AD among patients with type 2 diabetes. Previous work, including our own, has revealed expression of amyloid precursor protein (APP) in a variety of cells outside the brain including those exhibiting a secretory phenotype. Since pancreatic beta cells serve as the primary source of peripheral insulin secretion, we hypothesized that APP may play a role in pancreatic physiology. To begin testing this idea, four different mouse lines were compared; wild type, APP knockout, APP/PS1 and APP/PS1xAPP knockout. Western blot analysis revealed endogenous pancreas APP expression in wild type mice as well as significant over-expression of mutant human APP in the pancreas of both the APP/PS1 and APP/PS1xAPP knockout lines. APP immunoreactivity localized to the pancreatic islets with a dramatic increase in intensity in human APP over-expressing mice. However, there was no detectable immunoreactivity for Aβ in the pancreas nor any quantifiable increase in Aβ1-40 or Aβ1-42 via ELISA from the APP/PS1 and APP/PS1xAPP knockout lines. Comparison to human pancreas supported the murine findings demonstrating

robust APP immunoreactivity within the islets of both normal and diabetic pancreatic islets with no detectable Aβ immunostaining. These data support the idea that wild type or mutant APP may directly regulate pancreatic endocrine cell function perhaps contributing to the proposed insulin receptor signaling dysfunction hypothesized to occur in the brains of Alzheimer's disease patients.

Disclosures: J. Kulas: None. **C.K. Combs:** None.

Poster

406. APP and Abeta Pathology Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 406.29/F7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Amyloid precursor protein regulates microglial phenotype in a mouse model of alzheimer's disease

Authors: *G. D. MANOCHA, A. M. FLODEN, K. RAUSCH, L. ROJANATHAMMANEE, K. R. PUIG, K. L. PUIG, C. K. COMBS
Basic Sci., Univ. North Dakota, GRAND FORKS, ND

Abstract: Beta-amyloid peptide containing plaques are one of the major histopathological characteristics of Alzheimer's disease brains. Aβ peptides are derived from the proteolytic cleavage of the ubiquitiously expressed amyloid precursor protein (APP). Although much research has focused on APP cleavage products less effort has been spent studying APP itself. Our prior work demonstrated that APP can behave as a proinflammatory receptor on monocytic lineage cells mediating tyrosine kinase-based activation. Therefore, we hypothesized that it may serve a similar function in microglia. To test this idea, primary murine microglia cultures from wild type and APP knockout mice were stimulated with oligomeric or fibrillar Aβ. Although fibrillar Aβ stimulated increased protein phosphotyrosine levels and TNF-α secretion from both cultures, oligomeric stimulation of APP knockout microglia was significantly attenuated. Although APP knockout microglia cultures had no attenuation of phagocytic ability, they demonstrated significant impairment in migratory ability *in vitro* compared to wild type microglia. Similarly, intracerebroventricular infusions of oligomeric Aβ produced significantly less microgliosis in APP knockout compared to wild type mice. These findings suggested a role for APP in modulating the microglial response to selectively oligomeric Aβ. We next crossed APP knockout mice with mice expressing human mutant APP/PS1 in order to assess whether

absence of murine APP attenuated microgliosis in the presence of accumulations of Aβ oligomers and fibrils *in vivo*. The APP/PS1 x APP-/- mice had significantly reduced microgliosis in spite of similar plaque loads compared to APP/PS1 mice. This correlated with improved memory performance and decreased anxiety in the APP/PS1 x APP-/- mice. These data define a novel function for microglial APP in regulating their migratory ability and response to Aβ peptides which may be relevant for modulating their behavior in Alzheimer's disease.

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Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

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Program#/Poster#: 407.01/F8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the National Natural Science Foundation of China (81372107)

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Guangdong Province Science and Technology Department Project (2011B060300013)

Title: Effects of physical exercise on cognitive function: endothelial nitric oxide synthase contributing to Aβ accumulation in spontaneous hypertensive rats

Authors: L.-Y. ZHANG[1], J. LUO[1], Z. PEI[2], *R. A. ESPAÑA[4], W.-J. GAO[4], F. LI[3], X.-Q. HU[1]
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Abstract: Objective This study was aimed to examine whether the effect of physical exercise on nitric oxide synthase(eNOS) is benefit to decrease Aβ accumulation. Methods Twenty spontaneous hypertensive rats (SHR) were randomly divided into physical exercise hypertensive group (PE, n=10), which was given running wheel exercise for 3 months, and a sedentary hypertensive group (SED, n=10). Another 10 Wistar rats were used as normotensive group (NC, n=10) and they were fed in standard cages without any special training exercise. The blood pressure (BP) was monitored and measured every week. The rats were tested with Morris water maze for cognitive performance and were sacrificed for histological and biochemical study. The specific marker heme oxygenase -1(HO-1), endothelial eNOS, and β-site APP-cleaving enzyme

1(BACE1)/Aβ in endothelial cells cerebral cortex, and hippocampus were examined with immunofluorescence staining and western blotting analyses. Results BP in PE was significantly lower than that in SED ($p<0.05$), but was significantly higher compared to that in NC ($p<0.05$). Water maze performance in PE was significantly improved compared with that in SED ($p<0.05$), but was slightly and significantly worse than that in NC ($p>0.05$). Immunofluorescence analysis and western blotting assay in SED showed less HO-1/eNOS expression as compared to NC, but BACE1/Aβ accumulation was significantly up-regulated in endothelial cells, cerebral cortex, and hippocampus. After 12 weeks's training with physical exercise, there were significant up-regulation of HO-1/eNOS expression and down-regulation of BACE1/Aβ expression in endothelial cells, cerebral cortex, and hippocampus. Conclusions Physical exercise decreases BP and improves cognitive function in hypertensive rats through up-regulation of HO-1/eNOS, which may in turn decrease BACE1 expression and Aβ accumulation.

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Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.02/F9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC Grant 418489

CIHR Grant 126127

Title: Characterization of amyloid beta oligomeric composition using matrix assisted laser desorption ionization mass spectrometry

Authors: *J. S. WANG[1], K. JURCIC[2], S. WHITEHEAD[3], K. K. C. YEUNG[1]
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Abstract: Alzheimer's disease (AD) is currently the most prevalent neurodegenerative disorder, as well as the most common cause of dementia. Accumulating evidence supports the correlation between amyloid beta (Aβ) oligomers and severity of AD. Therefore, research emphasis has been placed on the analysis of amyloid-β aggregation and its influences on the pathology of AD. Aβ is known to aggregate and form soluble oligomers of various sizes, but the primary target of

AD research focuses on the major composition of amyloid plaques found in AD patients, peptides $A\beta_{1-42}$ and $A\beta_{1-40}$. Numerous protocols have been reported for *in vitro* oligomeric $A\beta_{1-42}$ preparations, yet the effect of variations among protocols on the resulting composition is poorly understood. Methods that provide qualitative and quantitative characterization of the monomeric and oligomeric Aβ species are therefore extremely important to oligomeric $A\beta_{1-42}$ research in AD progression and severity. The most common means of Aβ oligomer quantification is gel electrophoresis. However, it has low sample throughput, and gel smearing poses as a significant resolution limitation. Alternatively, the use of electrospray ionization mass spectrometry (ESI-MS) in conjunction with ion mobility (IMS) has been applied to study the assembly of Aβ oligomers. IMS is critical in differentiating oligomers of different sizes with the same m/z; circumvent the issue of overlapping m/z from non-covalent homo oligomers with multiple charge states from ESI. The research described here focuses on a simpler mode of MS based on matrix assisted laser desorption ionization (MALDI). MALDI produces predominantly singly-charged ions, allowing for oligomers of varying sizes to be resolved by a time-of-flight (TOF) mass analyzer. Our results demonstrate that MALDI-MS offers a fast and easy method to measure the extent of Aβ oligomerization. The method has been applied to determine the effect of protocol variations on the resulting composition of the Aβ assemblies.

Disclosures: **J.S. Wang:** None. **K. Jurcic:** None. **S. Whitehead:** None. **K.K.C. Yeung:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.03/F10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Evaluating the pathological and behavioural outcomes of amyloid beta oligomers in the rat

Authors: *R. WONG[1], D. F. CECHETTO[2], S. WHITEHEAD[2]
[1]Anat. and Cell Biol., [2]Western Univ., London, ON, Canada

Abstract: Alzheimer’s disease (AD) is the most common form of dementia and is characterized by a progressive neurodegeneration that ultimately leads to cognitive decline. AD typically occurs in the elderly, and with an aging population worldwide, it is projected to increase accordingly. Amyloid beta (Aβ) deposition is considered a key event in the progression of AD, yet Aβ can exist in many structural forms, and each form likely plays a different role during the development of AD. Recently, significant amounts of research have begun to focus on a soluble

oliogomeric form of A. *In vitro* studies have suggested that A oligomers may play a significant role in the progression towards AD from a normal healthy brain. Human studies have also shown the correlation between clinical diagnoses of AD with elevated levels of A oligomers. However, to date, there are few studies studying the effects of A oligomers in an *in vivo* model. The purpose of this project is to identify the spatial and temporal consequences of injecting A oligomers into the rat brain by evaluating pathological and behavioural outcomes. We hypothesize that injecting A oligomers into the rat brain will result in neurodegeneration, neuroinflammation that are concomitant with behavioural and cognitive deficits. A oligomers were characterized by western blot and injected either cortically or intracerebroventricularly into the rat brain. Survival time points were 1, 3, 7 or 21-days post surgery. Pathological using immunohistochemistry and behavioural analyses using the Morris Water Maze were done. Results demonstrate that A oligomers crossed into the parenchyma of the rat brain leading to a neuroinflammatory responses. Overall our results indicate that injection of A oligomers into the rat brain may be an alternative, non-transgenic strategy to investigate the effects of A oligomers in an *in vivo* model.

Disclosures: R. Wong: None. **D.F. Cechetto:** None. **S. Whitehead:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

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Program#/Poster#: 407.04/F11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The interaction between ZnT3 and Amyloid-β in Alzheimer's disease

Authors: *S. M. HANCOCK, D. I. FINKELSTEIN, A. I. BUSH, P. A. ADLARD
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Abstract: Introduction: Alzheimer's disease (AD) is the most common form of dementia for which there is currently no treatment or cure. Zinc has been repeatedly linked to AD with alterations in Zn distribution suggested to have a key role in AD. Moreover, a high binding affinity of zinc to amyloid-β (Aβ) has been demonstrated, along with the presence of Aβ plaques around glutamatergic synapses. Zinc transporter 3 (ZnT3) is responsible for loading Zn ions into presynaptic vesicles and is prevalent at glutamatergic synapses within the cortex. Past studies have shown that both ZnT3 mRNA and protein expression levels are altered in AD. We have demonstrated that ZnT3 is present in primary cultured mouse cells (neurons and astrocytes) and

expression levels can be influenced by treatments with zinc and copper. We have further examined the interaction between ZnT3 and Aβ *in vivo*. Method: Transgenic ZnT3 KO and WT mice at either 3 or 12 months of age were anaesthetised and Aβ was unilaterally injected (2ug/5µl in 0.02M PBS) into the right hippocampus (2.54mm from Bregma, lateral 2mm and 2mm depth). Behavioural testing was performed at 7 and 14 days post injection, with animals subsequently culled for biochemical and histological analysis. Conclusion: These analyses will further define the interaction between Aβ and ZnT3, providing insight into the pathogenesis of AD.

Disclosures: S.M. Hancock: None. **D.I. Finkelstein:** None. **A.I. Bush:** None. **P.A. Adlard:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.05/F12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: N-truncated and pyroglutamate-modified (pGlu) Aβ: On the molecular pathway of formation in Alzheimer's disease

Authors: *S. SCHILLING, D. SCHLENZIG, H. CYNIS, H.-U. DEMUTH
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Abstract: A significant proportion of amyloid-beta (Aβ) peptides in Alzheimer's disease (AD) is truncated at the N-terminus and may be post-translationally modified by pGlu. In particular, Aβ3pE-40/42 and Aβ11pE-40/42, have been shown to correlate with disease progression and being overrepresented in early-onset forms of inherited AD. Recently, we could show that Aβ3pE-40/42 form oligomeric structures with considerable surface hydrophobicity which, in turn, mediates a significantly enhanced neurotoxicity. The modification is formed by the enzyme glutaminyl cyclase (QC), which has been shown to be upregulated in AD. A competitive inhibitor of QC is going to enter Phase 2 clinical studies. On our quest to study the role and formation of N-truncated Aβ peptides, we characterized the processing of different APP proteins (wt or familial Alzheimer's mutations) in cell culture models. Using specific ELISA techniques, we could show that APP carrying the Swedish (APP695KM595,596NL]) mutation leads to generation of full-length Aβ (Aβ(1-40/42)), whereas processing of APPwt results in significant formation of N-truncated peptides suggesting alternative processing. Hence, we introduced

several mutations close to the primary β-secretase cleavage site of APP and could demonstrate suppression of BACEI - related Aβ but insignificant reduction of total Aβ formation. Among these constructs, processing of APP695[E599/Q] showed enhanced formation of pGlu-Aβ, which was not influenced or even slightly increased after BACE1-inhibition or expression in a BACE1-ko background. Co-expression of Meprin β, but not Meprin α, resulted in production of N-truncated Aβ peptides Aβ(2-40/42) and Aβ(3-40/42). These results were corroborated by cleavage of APP-related peptides by Meprin *in vitro*. Taken together, these data support that BACE-independent processing of APPwt contributes to formation of N-truncated Aβ. Several proteases have been suggested as potential APP-β site cleaving enzymes. Among such candidate enzymes of alternative processing routes, Processing of APPwt by Meprin can contribute to the formation of N-truncated Aβ. The truncated peptides might be prone to further N-terminal truncation or cyclization by glutaminyl cyclase. Therefore, meprin might represent a potential upstream target to suppress formation of pGlu-Aβ.

Disclosures: **S. Schilling:** None. **D. Schlenzig:** None. **H. Cynis:** None. **H. Demuth:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

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Program#/Poster#: 407.06/G1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Interaction of Alzheimer's β-amyloid peptides with cholesterol: Mechanistic insights into amyloid pore formation and inhibition by bexarotene

Authors: *C. DI SCALA, H. CHAHINIAN, N. GARMY, N. YAHI, J. FANTINI
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Abstract: Brain cholesterol plays a critical role in Alzheimer's and other neurodegenerative diseases. The molecular mechanisms linking cholesterol to neurotoxicity have remained elusive for a long time, but recent data have allowed to identifying functional cholesterol-binding domains in several amyloidogenic proteins involved in neurodegenerative disease, including Alzheimer's disease. We analyse the cholesterol-binding properties of β-amyloid (Aβ) peptides and the impact of these interactions on amyloid pore formation. We show that the cholesterol-binding domains of Aβ peptides and of its transmembrane precursor C99 are partially overlapping but involve distinct amino acid residues, so that cholesterol has a greater affinity for Aβ than for C99. Synthetic 22-35 and 25-35 fragments of Aβ retained the ability of the full-

length peptide 1-42 to bind cholesterol and to form zinc-sensitive, calcium permeable amyloid pores in cultured neural cells. Studies with mutant peptides allowed to identifying key residues involved in cholesterol binding and channel formation. Cholesterol promoted the insertion of Aβ in the plasma membrane, induced α-helical structuration, and forced the peptide to adopt a tilted topology that favoured the oligomerization process. Bexarotene, an amphipathic drug currently considered as a potential candidate medication for the treatment of neurodegenerative diseases, competed with cholesterol for binding to Aβ, and efficiently prevented oligomeric channel formation. These studies indicate that it is possible to prevent the generation of neurotoxic oligomers by targeting the cholesterol-binding domain of Aβ peptides. This original strategy could be used for the treatment of Alzheimer's and other neurodegenerative diseases that involve cholesterol-dependent toxic oligomers.

Disclosures: C. Di scala: None. **H. Chahinian:** None. **N. Garmy:** None. **N. Yahi:** None. **J. Fantini:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.07/G2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Development of an ex-vivo assay to measure solubility characteristics of plaque associated Aβ from human alzheimer disease (AD) frontal cortex

Authors: *J. A. TZAFERIS[1], H. B. OLUOCH[2], M. M. RACKE[2], J. T. HOLE[2], F. LIU[2], R. B. DEMATTOS[2]
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Abstract: Background: A large anti-Aβ study performed in aged PDAPP mice revealed differences in the Aβ composition of diffuse and cored plaques and suggested possible in-vivo solubility differences for different Aβ fragments. Histological analyses in the aged PDAPP mice indicated that BACE inhibitor treatment resulted in removal of diffuse plaque, whereas antibody treatment selectively lowered cored plaques. Furthermore, biochemical analyses of brain lysates revealed a greater lowering effect of the BACE inhibitor on Aβ 4-42, 9-42, and 11-42 as compared to the plaque-specific antibody treatment, thus suggesting an enrichment of these truncated peptides in diffuse plaque. Conversely, plaque-specific antibody treatment enhanced lowering effects on Aβ p3-42 and 8-42, suggesting enrichment in cored plaque. Based on these

findings, we sought to develop an ex-vivo assay to measure solubility characteristics of plaque associated Aβ species from human AD frontal cortex. **Methods:** Human AD frontal cortical sections (40 μm) were placed in 24-well plates and incubated in PBS containing 50μg/ml of a mid-domain antibody for 5, 30, 60, and 180 minutes. The antibody was incorporated to stabilize soluble Aβ in the PBS buffer to increase recoveries from non-specific binding. Following the specified times, the PBS supernatant and AD tissue sections (for normalization of PBS fraction) were analyzed by acid urea gels followed by Western blotting. Aβ levels were determined using antibodies selective for N-terminally truncated epitopes as well as full length Aβ. **Results:** The ex-vivo study in human cortical tissue identified differences in the solubilization of Aβ fragments associated with diffuse and cored plaques. We observed that Aβ species differentially diffused from the cortical tissue into the PBS buffer over time. Acid urea gel Westerns showed an increase of Aβ 4-42 (~1.5 fold), Aβ 1-42 (~2 fold), and Aβ 11-42 (~2 fold) levels in the PBS buffer over time, whereas, Aβ p3-42 and Aβ 8-42 levels remained constant. The maximal diffusion separation amongst Aβ analytes occurred at 30 minutes. Further analysis, at this time point, revealed a 1% diffusion of total Aβ 11-42, which is 4 fold greater than full length Aβ 1-42 and 10-30 fold greater than the rest of the N-terminally truncated Aβ fragments. **Conclusions**: We have identified plaque associated Aβ that solubilize from human AD frontal cortex with Aβ 11-42 exceeding the rest of the Aβ analytes. These findings parallel data obtained in aged PDAPP mice that suggested an enrichment of Aβ 11-42 in diffuse plaque and an increased solubility of Aβ 11-42 as compared to other N-terminally truncated Aβ fragments.

Disclosures: **J.A. Tzaferis:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **H.B. Oluoch:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **M.M. Racke:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.T. Hole:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **F. Liu:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **R.B. DeMattos:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.08/G3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG042890

NIH NIEHS Grant T32ES007254

Title: Modulated zinc levels may preserve insulin responsiveness in CNS synapses of non demented individuals who resist Alzheimer's Disease neuropathology

Authors: *M. M. COMEROTA[1], R. WOLTJER[3], G. TAGLIALATELA[2]
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Abstract: Alzheimer's disease neuropathology is characterized by deposits of aggregated amyloid beta (Aβ) and hyper-phosphorylated tau protein neurofibrillary tangles. In addition, the disruption of synaptic spines due to the binding of small Aβ oligomers on the post-synaptic density (PSD) is thought to be an early key event driving onset and progression of cognitive decline in AD. However, some individuals, here referred to as Non-Demented with Alzheimer's Neuropathology (NDAN), exhibit little to no cognitive decline despite the post mortem observation of plaques and tangles in the brain as seen in fully symptomatic AD, suggesting that NDAN individuals are resistant to the cognitive decline normally brought about by AD neuropathology. Understanding the mechanisms of such resistance would thus reveal important targets for the development of effective therapies for AD. Our previous results showed that several characteristics are associated with NDAN individuals that differ from demented AD patients, including absence of Ab oligomers at PSD, preserved insulin responsiveness and modulated levels of synaptic zinc. The goal of the current study was to investigate these characteristics and their relationship to the ability of Aβ oligomers to bind on to the PSD. Based on a previous report describing the inhibition of the autophosphorylation of the insulin receptor in the presence of 10 μM of zinc in a cell free system, in the current study, we investigated this phenomena in a whole cell system using human SY5Y neuroblastoma cells. Western blots were performed on total protein extracts from cells treated with 10 U of insulin in the presence of increasing concentrations of zinc; 10 μM, 100 μM, and 1mM. Our initial results indicated that also in a complex cell environment the presence of zinc inhibits the autophosphorylation of the insulin receptor, as well as, the associated signaling pathway (Akt and GSK-3β). These results were further compared to alterations in the binding of Aβ in SY5Y cells due to insulin resistance driven by elevated zinc levels. This study provides further understanding of the potential mechanisms associated with synaptic Aβ binding in the development of cognitive impairment which can lead to more effective therapeutic targets to prevent or slow cognitive decline associated with AD.

Disclosures: M.M. Comerota: None. **R. Woltjer:** None. **G. Taglialatela:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.09/G4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The inhibitory effect of Hsp60 on amyloid beta aggregation: A biophysical study

Authors: *C. MARINO[1,2], D. SPIGOLON[4], S. VILASI[4], R. PASSANTINO[4], M. R. MANGIONE[5], F. CAPPELLO[6], D. BULONE[5], G. TAGLIALATELA[3], P. SAN BIAGIO[5]
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Abstract: Alzheimer's disease (AD) is by far the most common neurodegenerative disorder whose incidence exponentially increases in the aging population. Although signs and symptoms of clinically manifest AD are well defined, no effective therapy is yet available able to regress it yet and the pathogenesis is still matter of discussion. One of the most accredited theory posits the crucial role in AD pathogenic pathways of amyloid beta peptide (Aβ), especially when organized as oligomers. These small Aβ aggregates are neurotoxic both *in vitro* and *in vivo*. In addition, Aβ oligomers have been shown to induce alterations of mitochondria and disrupt long term potentiation (LTP) in amyloid precursor protein (APP) overexpressing transgenic mice that recapitulate salient AD pathology. Despite this established experimental knowledge, potential mechanisms able to inhibit this cascade remain elusive. Compelling evidence by us and others indicates a mechanism of interaction between the Aβ peptide and the Hsp60, a mitochondrial chaperonin that may be involved in preventing mitochondrial deficits associated with AD pathology. Indeed, post mortem studies on AD brains revealed a significant increase of oxidative stress when compared to age-matched control subjects. Furthermore, there are combined and multiple variations in chaperones and proteasome activities, responsible for the accumulation of neurotoxic species in senescent central nervous system especially when AD occurs. While the involvement of Hsp60 in mitochondrial oxidative metabolism caused by Aβ is well documented, there are few studies describing the biophysical mechanism of direct interaction between these two proteins. Here, we investigate the inhibitory effect of Hsp60 on the Aβ1-40 aggregation by the Thioflavine T assay and Circular Dichroism analysis. Furthermore, Isothermal Titration Calorimetry experiments are conducted in order to characterize the Hsp60/ Aβ1-40 binding parameters. Our preliminary results show a direct interaction between Aβ1-40 and Hsp60 responsible for a significant slowdown in the aggregation kinetic and perturbation of the Aβ peptide secondary structure. Overall, these results suggest Hsp60 as a new target for the

development of innovative therapies for AD centered on prevention of Aβ-driven mitochondrial damage.

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Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.10/G5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Monitoring the early phase of beta-amyloid peptide aggregation using targeted and quantitative mass spectrometry

Authors: ***A. W. SCHMID**[1], N. RUDINSKIY[2], D. DEMURTAS[1], M. MONIATTE[1]
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Abstract: Soluble beta-amyloid (Abeta) oligomers account for the decline in synaptic plasticity and memory associated with dementia and therefore represents an important, early diagnostic and therapeutic target for the treatment of Alzheimer's disease (AD). In order to elucidate the mechanisms of peptide misfolding in the early stages of AD pathogenesis, it is important to develop sensitive and specific assays to identify and monitor key molecular triggers and biological markers before the development of clinical symptoms. We are interested in monitoring key molecular changes associated with the early stages of Abeta peptide oligomerization using high resolution mass spectrometry (MS) analysis. In this project we have investigated the early events of Abeta peptide folding using targeted and quantitative mass spectrometry and revealed that, Abeta peptide aggregation is associated with the generation of a typical peptide fragment fingerprint. These findings have enabled us to design and develop a new type of anti-Abeta antibodies, which specifically target key molecular changes associated with the early events of peptide oligomerization and therefore serve as a novel and indispensable analytical tool for monitoring peptide aggregation *in vitro* and *in vivo*. Screening of transgenic mice and human post-mortem AD brain tissue, using a combination of immunoprecipitation and quantitative MS revealed that these molecular changes are highly relevant events found *in vivo*. We believe that this observation provides novel and crucial information for quantifying absolute

Abeta levels in biological samples, such as CSF and plasma, as well as for understanding and targeting the inhibition of protein aggregation.

Disclosures: **A.W. Schmid:** None. **M. Moniatte:** None. **D. Demurtas:** None. **N. Rudinskiy:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

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Program#/Poster#: 407.11/G6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Acumen Pharmaceuticals, Inc.

NIH grant EB000768

Title: Oligomeric Aβ-induced neuronal toxicity is attenuated by ADDL-specific antibodies *in vivo*

Authors: ***X. WANG**[1], M. ARBEL-ORNATH[1], J. JERECIC[2], G. A. KRAFFT[2], B. J. BACSKAI[1]
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Abstract: Alzheimer's disease (AD) is characterized by aggregation of amyloid-β (Aβ) and tau protein along with progressive dementia. Soluble Aβ oligomers (sAβo) / Aβ-derived diffusible ligands (ADDLs) have been shown to be toxic for neurons, and they are believed to be key factors in synaptic degeneration and memory loss in AD. We sought to investigate the molecular mechanisms that couple ADDLs to dysfunction of neuronal networks. In this study, we used array tomography, a technique that allows precise quantification of synapses based on ultrathin tissue sectioning and immunohistochemistry, to quantify the reductions of synaptic density in the presence of sAβo in transgenic mice and human AD tissues and we observed a significant decrease in postsynaptic density associated with microdeposits of oligomeric Aβ. In primary neuronal cultures, application of Tg2576 conditioned media or synthetic ADDLs led to an increase in intracellular calcium that was prevented with immunodepletion using the soluble Aβ oligomer selective antibody ACU-3B3. Furthermore, we used multiphoton imaging in wildtype mice to measure neuronal calcium in individual cortical neurons *in vivo* by using the genetically

encoded ratiometric calcium indicator Yellow Cameleon 3.6. After topical application of synthetic ADDLs (5 nM) onto the exposed cortical surface, the average intracellular calcium levels in layer 5 neuronal soma increased by ~1.2-fold compared to resting calcium levels. Pre-treatment with ACU-3B3 (1ng/ml) significantly reduced the ADDL-induced elevation in intracellular calcium. These data demonstrate that ACU-3B3, the murine precursor of humanized antibody ACU-193, selectively inhibits the toxicity of sAβo, and is able to restore neuronal calcium homeostasis *in vitro* and *in vivo*.

Disclosures: X. Wang: None. **M. Arbel-Ornath:** None. **J. Jerecic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acumen Pharmaceuticals, Inc. **G.A. Krafft:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acumen Pharmaceuticals, Inc.. **B.J. Bacskai:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT CB2011-169023

VIEP-BUAP-NAT-Hc-2014

Aleidy Patricio is scholarship CONACyT-350139

Title: The injection of the beta amyloid peptide 25-35 in magnocelular nucleus decreases learning and spatial memory associated with the inflammatory process and decrease signaling Trk-A receptor

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Abstract: Alzheimer´s disease (AD) is a syndrome of dementia, characterized by gradual degeneration of the basal-forebrain, cholinergic neurons innervating the cortex, amygdala, and hippocampus. In brains of AD patients the aggregation and accumulation of β-amyloid protein

(Aβ) contributes to cholinergic-neuronal degeneration, in turn causing learning and memory deficits. In particular, basal forebrain cholinergic cells have been found to be selectively vulnerable to the consequences of inflammation, suggesting that Aβ exerts its degenerative effect on cholinergic cells. The injection of Aβ25-35, the biologically active fragment, causes axonal and dendritic retraction followed by neuronal death. In recent years it has been shown that activation of the Trk-A receptor as white neurotrophins NGF has an essential role in cholinergic function. Trk-A downstream signalling is activated, and that the enhancement of Akt pathway leads to GSK-3β inhibitory phosphorylation. Given that GSK-3β inhibition reduces its ability to phosphorylate Tau, the TrkA/Akt activation may be interpreted as a response against the Aβ. In the present study, our aim was to evaluate the effects of the injection of the Aβ25-35 peptide in the magnocellularis nucleus on spatial memory, the GFAP protein, Trk-A receptor, GSK-3β and Akt. Male Wistar rats (270 g) were injected with 1 μL of Aβ35-25 [1 mM] (control group) in the magnocellularis nucleus. In a second group was injected with 1 μL of Aβ25-35 [1 mM]. The learning assessment was made in the eight-arm radial maze at 20 days after injection. The memory test was done ten days after the learning assessment. The immunoreactivity of GFAP and the evaluation of TrkA receptor, GSK-β and Akt by western blot were performed in in the magnocellularis nucleus, frontal cortex and temporal cortex (TCx, FCx). Our findings show that Aβ25-35 into the magnocellularis nucleus induces a reactive gliosis in the TCx and FCx at 1, 5, 10, 15 and 32 days after injection. Thirty days after Aβ25-35 injection we found that phosphorylation of TrkA receptor was decreased in the magnocellularis nucleus without changes in the CtxT. On another hand, we found an increment in the activity of GSK-3β phosphorylated at Tyr 216. Finally behavioral performance showed that the neurodegeneration evoked by Aβ25-35 delayed acquisition of learning and memory. These results show that the inflammatory reaction probably contributes degenerative process and induce dysfunction in the signaling TrkA receptor.

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Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

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Program#/Poster#: 407.13/G8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG-00538

AG-00096

Title: Effects of Interleukin-1 and β-Amyloid on endosomal and endocytic function in neurons

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Abstract: Inflammation plays both a protective and damaging role in Alzheimer disease (AD). To identify a long-lasting and effective treatment, it is important that we better understand its underlying processes in which chronic inflammation exerts deleterious effects in the brain. Our studies implicate a critical pro-inflammatory cytokine, interleukin-1β (IL-1β), as a factor that accelerates AD pathology via fundamental alterations to endosomal function. Pro-inflammatory molecules and beta amyloid accumulate in the brain with age and in AD, which is linked to impaired neuronal function and poor cognitive outcomes. Proper endosomal sorting is critical for many intracellular signaling systems and impaired endosomal function in neurons may be an underlying mechanism driving poor cognitive outcomes. In this study, we describe the mechanisms by which beta amyloid (Aβ) accumulation and inflammation (specifically IL-1β) impair plasticity and neuronal health. We have found that these molecules interfere with endosomal trafficking of brain-derived neurotrophic factor (BDNF) and its receptor TrkB, leading to impaired neuronal health, function and synaptic plasticity. Our data reveal that IL-1β and Aβ both interfere with retrograde axonal flow of TrkB/BDNF, impair events downstream of TrkB/BDNF signaling, and fundamentally alter cell-surface receptor endocytosis. These data indicate that Aβ and IL-1β deteriorate synapses by targeting both pre- and post-synaptic components, impairing signal transduction and synaptic plasticity and placing neurons at risk for degeneration. We propose that impairment of endosomal trafficking is a common mechanism by which Aβ and IL-1β impair neuronal signaling and plasticity. Supporting the hypothesis that dysfunctional endosomal trafficking is an important contributor to AD pathogenesis, several recently identified genetic risk factors for AD impact the function of endocytotic pathways (e.g., PICALM, BIN1, CD2AP). At present, we are currently investigating the interactions and implications of AD genetic risk factors as contributors to endosomal sorting and endocytic pathways. Ideally, our goal is to understand the nature of the interaction between AD, inflammation and endosomal function to determine if select intervention strategies can protect against the detrimental effects of IL-1β on endosomal sorting and cognition.

Disclosures: A.J. Carlos: None. **C.W. Cotman:** None.

Poster

407. Amyloid Beta Aggregation and Toxicity

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 407.14/G9

Topic: C.05. Aging

Support: R21NS067335

NS070577

Frazier Institute

Title: A therapeutic miRNA for brain disorders

Authors: *K. C. SONNTAG, W. KIM, Y. LEE, D. MCPHIE, K.-S. KIM, B. COHEN
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Abstract: One of the main hurdles in developing novel therapeutics for age-related disorders is the still limited understanding of both the biology of normal aging and the mechanisms of disease pathology. Aging and age-related disorders are characterized by slow progressive deterioration or death of neurons, and are influenced by age- and disease-specific factors, including genetic predisposition, dysfunctional proteins, and compensatory mechanisms and molecules that are important in cell survival. If the cellular defense mechanisms are compromised, the penetrance of disease-specific mechanisms becomes higher and cell survival less likely. Among the regulatory factors that govern gene and protein networks and, consequently, influence neuronal health and function are small molecules such as miRNAs. It is increasingly appreciated that even small disturbances of these regulatory factors can have profound effects on cell survival in response to stress. We have identified a novel mechanism in neurons, mediated by miR-126, which regulates the effects of numerous neurotrophic and neuroprotective growth factors (GF). Specifically, we found that elevated levels of this miRNA are neurotoxic and increase the vulnerability of neurons to a variety of non-specific and disease-specific toxic factors, including Staurosporine (STS), Alzheimer's disease (AD)-associated amyloid beta 1-42 oligomers (Aβ1-42), and 6-OHDA which induces oxidative stress in dopamine (DA) neurons mimicking Parkinson's disease (PD) pathology. Mechanistically, miR-126 targets a series of factors in PI3K/AKT/GSK-3β and MAPK/ERK signaling pathways and small increases of this miRNA cause a downregulation of these signaling cascades, impairing the effects of neurotrophic and neuroprotective GF, such as IGF-1, NGF, BDNF, and soluble amyloid precursor protein α (sAPPα). In turn, inhibiting miR-126 enhances the actions of GF without disturbing normal neuronal cell function. Our data indicate that miR-126 may play a profound role in neuronal cell survival, at least in part by regulating GF/PI3K/AKT and MAPK/ERK signaling. While its elevation is neurotoxic, its inhibition is neuroprotective, suggesting that targeting this miRNA may have therapeutic potential for neurological and age-related disorders.

Disclosures: **K.C. Sonntag:** None. **W. Kim:** None. **Y. Lee:** None. **D. McPhie:** None. **K. Kim:** None. **B. Cohen:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.01/G10

Topic: C.02. Alzheimer's Disease and Other Dementias

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DOD Grant W81XWH-13-1-0384

Alzheimer's Association NIRG

Title: Impact of ApoE polymorphisms on amyloid-beta oligomerization and associated cognitive decline

Authors: ***E. L. CASTRANIO**, N. F. FITZ, A. Y. CARTER, I. LEFTEROV, R. KOLDAMOVA
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Abstract: Recent studies suggest amyloid beta (Aβ) oligomers as the major source of pathogenicity in Alzheimer's disease (AD), as opposed to the characteristic senile plaques in the brain. It has been shown Aβ oligomers contribute to inhibition of long-term potentiation, synaptic dysfunction and overall cognitive decline. Inheritance of the APOEε4 allele, as opposed to APOEε3 or APOEε2, is the only recognized genetic risk factor for sporadic late-onset AD, increasing risk by up to twelve-fold. Furthermore, it has been proposed that apolipoprotein E (APOE) impacts the oligomerization process and clearance of these oligomers from the brain and these functions appear to be dependent on the lipidation state of APOE. The fact that APOE4 is poorly lipidated and found at significantly lower concentration than APOE3 could contribute to the overall oligomeric pathology and risk for AD. The goal of the current study is to determine

how equal concentrations of human APOE3 or APOE4 impact Aβ oligomerization and associated cognitive decline. Oligomers produced in the presence of the different APOE particles were first characterized by electron microscopy, A11 specific dot blot, and western blot. Three-month old wild-type mice received implantations of guide cannulas into their hippocampus. Prior to cognitive tests of novel object recognition and fear conditioning, the mice received an infusion into the hippocampus of either Aβ oligomers or scrambled Aβ peptide in combination with APOE3 or APOE4. Preliminary data shows that co-incubating Aβ oligomer with APOE3 reduces the levels of high molecular weight oligomers in EM pictures and infusion of APOE3 with Aβ oligomers into the hippocampus prevents learning deficits in cognitive testing compared to Aβ oligomers alone. This study shows a comparison of co-incubation of Aβ oligomers with APOE3 versus APOE4 and demonstrates the impact of APOE genotype and concentration on learning and Aβ oligomer induced pathology.

Disclosures: **E.L. Castranio:** None. **N.F. Fitz:** None. **A.Y. Carter:** None. **I. Lefterov:** None. **R. Koldamova:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.02/G11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Drug Discovery Foundation

Title: Gene delivery of apoE2 reduces brain amyloid burden in apoE4-target replacement APP.PS1 transgenic mice

Authors: ***L. ZHAO**[1], A. J. GOTTESDIENER[2], M. PARMAR[1], M. LI[4], S. M. KAMINSKY[3], M. J. CHIUCHIOLO[3], D. SONDHI[3], D. M. HOLTZMAN[5], R. G. CRYSTAL[3], S. M. PAUL[1]
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Abstract: Apolipoprotein E4 allele (APOE4) carriers have a markedly increased risk for developing Alzheimer disease (AD) whereas the APOE2 allele is protective, reducing AD risk by 50% and markedly delaying the age of onset. ApoE is also a major determinant of amyloid β-peptide (Aβ) deposition and brain amyloid burden in AD patients and transgenic mice

(E4>>E3>E2). We have previously reported that lentiviral-mediated gene delivery of apoE2 significantly reduces brain Aβ levels and amyloid burden in APP transgenic mice. We have now extended this finding using adeno-associated viral (AAV) vectors and a new APP.PS1/TRE4 mouse model where Aβ/amyloid deposition is dependent on apoE4 expression. We administered 0.25X1010, 0.5X1010, or 1X1010 viral genomes (vg) of AAVrh10-CAG-E2 bilaterally into hippocampi of young (2.5-month old) APP.PS1/TRE4 mice (mild to moderate amyloid pathology in hippocampus). Using specific ELISAs to quantify apoE and Aβ levels in brain, we observed an apoE dose-dependent reduction in Aβ 8 weeks post injection; however, when 1X1010 vg virus were delivered via the same route to 5-month old APP.PS1/TRE4 mice (≥ 8 times more Aβ in their hippocampi), there was no statistically significant reduction in Aβ burden even with apoE2 levels that are comparable in both groups of mice. This suggests that the "efficacy" of apoE2 gene delivery in reducing brain Aβ/amyloid burden may depend on the degree of preexisting amyloid pathology. We therefore defined an apoE2/Aβ42 ratio (apoE2 levels in E2 injected mice/Aβ42 levels in control mice) to assess the effectiveness of treatment. We calculated that the apoE2/Aβ42 ratio in effectively treated young APP.PS1/TRE4 mice was 17.8 but was only 3.7 in older (5-month old) APP.PS1/TRE4 mice, where pathology was already quite advanced. We next administered AAV9-CAG-E2 intrathalamically in young (2.5-month old) APP.PS1/TRE4 mice. ApoE2 levels were increased 7.5-fold in the thalamus, 2.6-fold in the hippocampus, 1.7-fold in the cortex. Correspondingly, Aβ42 levels were decreased 70% ($p < 0.001$) in the thalamus, 45% ($p < 0.05$) in the hippocampus, and 14% ($p > 0.05$) in the cortex. The apoE2/Aβ42 ratios in these same three brain regions were 32.7, 7.2, and 2.4, respectively, demonstrating a strong positive correlation between the level of apoE2 expression and the reduction in tissue Aβ42 levels. Taken together, our study confirms that AAV-mediated apoE2 gene delivery reduces brain Aβ burden in transgenic mice where the latter is dependent on apoE4 expression. Importantly, the level of apoE2 expression and the degree of already existent amyloid pathology were important determinants of the "efficacy" of apoE2 gene therapy in this model.

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Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Investigator Initiated Research Grant (133086)

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Title: ApoE4 and ApoE4 domain interaction induce gender vulnerability on progression of Alzheimer's disease in mouse models

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Abstract: The increased prevalence and severity of Alzheimer's disease (AD) in women is strongly associated with age-related ovarian hormone loss and apolipoprotein E 4 allele (ApoE4), which is the most important genetic risk factor for sporadic AD. The interaction between ApoE4 and sexual hormone and how they interact in AD are still unclear. To evaluate the contribution of ApoE4, and specifically ApoE4 domain-interaction, on gender-dependent AD progression, we used three mouse models for AD: 3xTg AD mice bearing three familial AD mutations (APPSwe, PS1M146V, tauP301L); 3xTgAD with the human ApoE4 allele (Apoe4/3xTgAD); Mice with Arg-61 mutation that specifically exhibits only the domain interaction feature of human ApoE4. The transgenic mice were compared with age and gender matched nonTg background control mice. The results showed that all the transgenic mice showed learning and memory deficits compared to gender and age matched controls, with more severe in female ApoE4 transgenic mice. In radial arm water maze (RAWM) test, female ApoE4/3xTg mice took longer time and exhibited more errors to find the target than male ApoE4/3xTg mice and female 3xTg mice. Female ApoE4/3xTg mice also failed to locate the target arm during the probe trail of RAWM. In novel arm discrimination (NAD) test, only female 3xTg and ApoE4/3xTg mice failed to locate the novel arm. Interestingly, female Arg61 mice also took longer time and more errors to find the target than female C57BL/6J mice and male Arg61 mice; Only female Arg61 mice failed to locate the target arm during the probe trail of RAWM after two days training, and to recognize the novel arm in NAD. In the lacking of ovarian hormones (ovaries were removed by ovariectomy (OVX)), ApoE4/3xTg and Arg61 female mice showed more severe deficits to locate the target arm or the novel arm in RAWM and NAD, respectively. The OVX effect was not observed in nonTg or C57BL/6J background mice. Pathologically, amyloid precursor protein (APP) and amyloid β (Aβ) oligomer expression was significantly higher in female ApoE4/3xTg and female Arg61 mice than in male. OVX further increased APP and Aβ oligomer protein expression in the hippocampus in female

ApoE4/3xTg AD. Our study suggests that the ApoE4 allele, especially the domain interaction in ApoE4, play a pivotal role in gender dependent development on AD both in cognition and brain AD pathology, particularly in females in the estrogen-deficiency condition. This study will also provide several potential therapeutic strategies for AD therapy in females.

Disclosures: X. Hou: None. **S.O. Adeosun:** None. **X. Zhao:** None. **B.R. Barlow:** None. **B. Zheng:** None. **M.J. Brents:** None. **Q. Zhang:** None. **J. Wang:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.04/H1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of an astrocytoma-enriched human liver X receptor alpha (LXRα) isoform

Authors: *M. BEYNA[1], H. S. XI[2], R. Y. YANG[2], E. K. SYLVAIN[1], N. POZDNYAKOV[1], W. L. BLAKE[3], G. RAMASWAMY[1]
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Abstract: Cholesterol is a vital component of cell membranes and myelin and is important for synaptogenesis and optimal neurotransmitter release. Because the blood-brain barrier inhibits lipid exchange with the periphery, almost all brain cholesterol is locally synthesized. Disturbances in the tight control of brain cholesterol homeostasis are linked to neurodegenerative disorders including Alzheimer’s disease (AD). Liver X receptor alpha (LXRα) is a master transcription factor regulating cholesterol homeostasis. One of its target genes is apolipoprotein E (apoE), a key cholesterol transport protein in the brain with critical roles in neuronal repair, synaptogenesis and clearance of amyloid-β peptide. One of the human apoE isoforms, apoE4, is associated with a strong risk for developing sporadic and late-onset forms of AD. LXRα activation upregulates apoE expression and secretion in astrocytes. Several LXRα isoforms arising from alternative splicing and differential promoter usage have been reported and are implicated in differential LXRα signaling and regulation. Given the importance of lipid metabolism in the brain, we investigated the presence and expression profiles of LXRα variants in the brain and different cell types. We utilized the Genotype-Tissue Expression database to investigate the possibility of alternatively spliced human LXRα gene transcripts. We identified a

novel transcript variant obtained from alternative splicing of exon 1 to exon 5. Compared to the major lipid-regulatory LXRα isoform, this new splicing event would result in a significantly smaller protein. This shortened isoform would lack the activation function 1 domain, DNA binding domain, and a part of the hinge region but retain the ligand binding and activation function 2 domains. Interestingly, the tissue distribution of the new splice junction frequency was significantly higher in the brain relative to other tissues, with the frequency in the liver being very low. We designed transcript-specific qPCR primers and measured the mRNA abundance of the isoforms in several human cell types: primary cortical astrocytes, astrocytoma and hepatocellular carcinoma cell lines, macrophage-like cells differentiated from a monocyte cell line, and primary peripheral blood mononuclear cells. The relative expression of the LXRα exon 1-5 splice variant was higher in the astrocytoma cells than that in the hepatocellular carcinoma cells. Further investigations will explore potential functions of this new isoform. Our results suggest that we have identified a new, putative LXRα splice variant with high expression levels in the human astrocytoma cells relative to that in a hepatocyte cell line.

Disclosures: **M. Beyna:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **H.S. Xi:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **R.Y. Yang:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **E.K. Sylvain:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **N. Pozdnyakov:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **W.L. Blake:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc. **G. Ramaswamy:** A. Employment/Salary (full or part-time):; Pfizer, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.05/H2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Arizona Alzheimer's Consortium

Midwestern University

Title: Mitochondrial peptide accumulation in cortical cytosolic compartments of APOE4(+) young adults

Authors: *M. PERKINS[1,2], D. SHONEBARGER[1], L. HENDERSON[1], J. VALLA[1,2]
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Abstract: The mechanism by which the apolipoprotein E ε4 (APOE4) allele increases risk for development of late-onset sporadic Alzheimer's disease (AD) is not known. However, young adult carriers of this genetic risk factor display significant functional deficits in cerebral glucose metabolism as well as mitochondrial oxidative metabolism, with features similar to AD patients. These functional changes in young adults were apparent in the absence of any significant changes in AD-related neuropathology such as amyloid or tau deposition. Recently, variants of another gene in linkage disequilibrium with APOE, TOMM40, which encodes the pore-forming subunit of the protein translocase of the outer mitochondrial membrane, were identified as a putative risk factor for late-onset AD. To determine whether APOE4 related to mitochondrial translocase function, we analyzed the levels of 4 nuclear-encoded mitochondrial proteins and 1 mitochondrially-encoded electron transport chain (ETC) protein in cortical lysates and in isolated mitochondria. Subjects consisted of 18-40 year-old APOE4 carriers (N=12) and APOE4 noncarriers (N=12). Frozen posterior cingulate/precuneus cortex samples were received from the NICHD Brain and Tissue Bank for Developmental Disorders (University of Maryland, Baltimore). A portion of the cortical block was homogenized in RIPA buffer. A second portion was homogenized in a sucrose-Tris-ATP buffer, the mitochondria were labeled with anti-TOMM22 paramagnetic beads, and then isolated in a magnetic field (Miltenyi Biotec). Both samples were similarly solubilized in RIPA buffer and subjected to SDS-PAGE and Western blotting. An antibody cocktail against select subunits of mitochondrial oxidative phosphorylation (Complexes I-V) was applied. In the isolated mitochondria, in accordance with the functional declines reported previously, each of the ETC subunit levels trended lower in APOE4(+) subjects (mean decline =17%). In stark contrast, the whole-tissue lysates from the same APOE4(+) subjects showed highly significant (mean increase =138%; $P<0.05$) increases in these same markers. While these findings can be interpreted in the context of mitochondrial protein translocation (i.e., a TOMM40 functional defect), the mitochondrially-encoded protein subunit showed the same effect. Thus, these results may be due to another mechanism. Nuclear pseudogenes for ETC proteins have been identified, and their overexpression and translation could explain these results. However, the rationale for such significant mitochondrial protein overexpression in the cytosolic compartments of APOE4(+) subjects remains unknown.

Disclosures: **M. Perkins:** None. **D. Shonebarger:** None. **L. Henderson:** None. **J. Valla:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.06/H3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R37NS34467

RO1AG039452

R37AG23084

Title: Apolipoprotein E isoforms differentially regulate blood-brain barrier breakdown in Alzheimer's disease

Authors: ***M. R. HALLIDAY**[1], Q. MA[2], Z. ZHAO[2], C. A. MILLER[3], B. V. ZLOKOVIC[4]
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Abstract: In humans, apolipoprotein E (apoE) has three isoforms: apoE2, apoE3, and apoE4. APOE4 is a major genetic risk factor for Alzheimer's disease (AD), a debilitating dementia characterized by early and progressive neurovascular dysfunction. Apolipoprotein E4 has direct effects on the cerebrovascular system, resulting in microvascular lesions and blood-brain barrier (BBB) damage. Our recent studies in transgenic mice have demonstrated that apoE4 leads to BBB breakdown by activating the proinflammatory cyclophilin A (CypA)-matrix metalloproteinase-9 (MMP-9) pathway in brain pericytes, which in turn results in degradation of BBB tight junction and basement membrane proteins. In this study, we analyzed post-mortem tissue from AD individuals with different APOE genotypes to determine whether apoE4-dependent activation of the CypA-MMP-9 pathway and resulting BBB breakdown as shown in APOE4, but not APOE3 or APOE2 transgenic mice, also occur in humans. Here, we show increased activation of the CypA-MMP-9 pathway in AD individuals compared with neurologically intact controls and that activation of the pathway is regulated in an APOE4 gene dose-dependent manner. We also show for the first time that cortical pericyte populations are reduced in AD individuals in an APOE4 gene dose-dependent manner and that reductions in

brain pericyte populations in AD significantly correlate with the magnitude of BBB breakdown as measured by extravascular accumulation of plasma proteins.

Disclosures: **M.R. Halliday:** None. **Q. Ma:** None. **Z. Zhao:** None. **C.A. Miller:** None. **B.V. Zlokovic:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.07/H4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation A2012421

Title: Inhibition of Inducible Degrader of LDLR (IDOL) markedly increases extracellular Aβ clearance by astrocytes

Authors: ***J. KIM**[1], D.-E. CHUNG[2], H. YOON[2], J. KIM[2]
[1]Dept. of Neurol., Mayo Clin. Birdsall Rm216, Jacksonville, FL; [2]Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Accumulation of Aβ in the brain has been hypothesized to be the primary cause of Alzheimer's disease (AD). Prevailing evidence suggests that Apolipoprotein E (APOE) genotype, the strongest genetic risk factor for AD, plays critical roles in Aβ metabolism and AD pathogenesis. Previously, we and others have demonstrated that genetic deletion of Apoe markedly decreases Aβ deposition in the brain of Aβ-amyloidosis mouse models. Moreover, we have shown that overexpression of the low density lipoprotein receptor (LDLR), a major apoE receptor in the brain, markedly decreases apoE level and Aβ deposition in the brain of Aβ-amyloidosis mouse model by increasing extracellular Aβ clearance by astrocytes. Therefore, understanding the molecular mechanisms underlying apoE and apoE receptor metabolism in the brain is likely to provide better insights into Aβ metabolism and AD pathogenesis. Recently, inducible degrader of the LDLR (IDOL), an E3 ubiquitin ligase, has been identified as a novel regulator of LDLR in the periphery that ubiquitinates and targets LDLR for degradation. However, our understanding of IDOL function in the brain is limited and whether IDOL influences Aβ metabolism has not been investigated. Interestingly, IDOL is expressed in the brain as well as periphery. Moreover, IDOL is widely expressed in the brain and is well detected in both neurons and glial cells. Therefore, we hypothesized that IDOL may play a critical role in

Aβ metabolism by regulating LDLR in the brain. As in peripheral cells, overexpression of IDOL increases ubiquitination of LDLR and decreases LDLR level, while RNAi-mediated inhibition of IDOL dramatically increases LDLR level in neuronal cells. Moreover, inhibition of IDOL decreases extracellular apoE level in primary astrocyte culture. Furthermore, inhibition of IDOL markedly increases extracellular Aβ clearance by astrocytes. These findings suggest that inhibiting IDOL function in the brain may represent a novel therapeutic strategy for AD.

Disclosures: J. Kim: None. **J. Kim:** None. **D. Chung:** None. **H. Yoon:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.08/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: ApoE dependent epigenetic regulation in brain: ApoE3 exports and ApoE4 imports HDAC4 and 6 to the nucleus

Authors: *A. SEN, D. L. ALKON
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Abstract: Apolipoprotein E4 (ApoE4) is a major genetic risk factor for sporadic Alzheimer's disease (AD). ApoE 4 also increases the risk of cerebral amyloid-angiopathy, age-related cognitive decline, Parkinson's and multiple sclerosis. Abnormal acetylation of histones is involved in the pathology of AD. Abnormal acetylation of histones is involved in AD. Here we show for the first time the involvement of ApoE in epigenetic regulation through histone deacetylases (HDACs). ApoE3 increased histone 3 acetylation than ApoE4 in SH-SY5Y cells. ApoE4+Chol induced a 2-fold increase in nuclear import of HDAC6 (47.2 ± 5.6%) compared to ApoE3+Chol (28.8 ± 3.1%), HDAC4 also showed a significant increase in nuclear translocation in ApoE4+Chol-treated cells (55.3 ± 1.4%) compared to ApoE3+Chol (32.4 ± 3.8%; t-test, P=0.005). Nuclear abundance of HDAC4 & 6 was higher in the hippocampus of ApoE4 transgenic mice than ApoE3 and control. ApoE3 prevented amylosheroids (ASPDs) induced nuclear import of HDACs. Blocking ApoE receptors by receptor associated protein (RAP) abolished the ApoE effect on HDACs. ApoE3 activated protein kinase C epsilon (PKCε) and PKCε overexpression retained HDACs in cytosol. PKCε activation reversed the ASPD-ApoE4 induced nuclear import of HDACs. These data suggest that ApoE4 may suppress vital neuronal gene expression by nuclear translocation of HDAC4 & 6 via deactivation of PKCε and PKCε

activation reverses the condition, thus PKC activators along with HDAC inhibitors might be a suitable therapeutic option for AD.

Disclosures: A. Sen: None. **D.L. Alkon:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

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Program#/Poster#: 408.09/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant PO1AG030128

Title: Apoplipoprotein E modulates activation of the extracellular signal-regulated kinase in response to Amyloid pathology

Authors: *Q. LI, A. LUSSIER, E. J. WEEBER
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Abstract: Apolipoprotein E (ApoE) modulates extracellular signal-regulated kinases (ERK) signaling transduction and may contribute to the functional difference of apoE isoforms on the development of Alzheimer disease (AD). To test this idea, we first detected changes of ERK activation by acute application of amyloid β42 oligomer (AβO) on brain hippocampal slices from 3-month old apoE target replacement (TR) mice, which express one of three human apoE isoforms (ApoE2, ApoE3, and ApoE4),by using Western blotting. There was an increase in ERK activation (phosphorylation form) in slices of apoE4 TR mice but not in the apoE2 TR and apoE3 TR mice following acute exposure to the AβO (2μM) for 30 and 60 minutes. We next sought to determine how the apoE isoforms influence ERK activation in response to the deposition of amyloid β plaques in brain of the apoE TR/5xFAD mice, which co-express one of the human apoE isoforms and 5 mutant AD familial genes leading to a large Aβ42 load at an early age. Using Western blotting and immunohistochemistry, we found a significant increase in ERK activation in the CA1 region of hippocampus in the apoE4 TR/5xFAD when compared with the apoE2 TR/5xFAD and apoE3 TR/5xFAD mice. Intriguingly, the activated ERK was largely seen in the astrocytes in the subventricular zone in the apoE4 TR/5xFAD mice compared to the apoE3 TR/5xFAD mice. Taken together, our data indicate that apoE modulates ERK signaling in an isoform dependent manner in response to amyloid pathology and may contribute a novel mechanism of the apoE isoform-dependent development of AD.

Disclosures: **Q. Li:** None. **A. Lussier:** None. **E.J. Weeber:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.10/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: IDOL inhibitors offer the potential for the treatment of Alzheimer's Disease

Authors: S. KUMAR, *T. R. BUTT, M. EDDINS, J. WU, J. LAROCQUE, S. AGARWAL, J. MARBLESTONE, M. KODRASOV, B. NICHOLSON
Progenra Inc, Malvern, PA

Abstract: Progressive loss of cognitive skills including memory is a hallmark of neurodegenerative diseases, e.g. Alzheimer's (AD); mild cognitive impairment is an early sign of AD. More than 5 million Americans have AD and the number is expected to grow as the percentage of the population over age 65 increases, posing a huge healthcare challenge . There is no cure for AD; the few FDA approved drugs treat symptoms, have serious side effects, and work in only 50% of patients. Due to the underlying complexity of AD, several different approaches are needed for effective management. Due to the significant unmet need, various groups are seeking to develop drugs to ameliorate cognitive deficits associated with neurodegenerative disease. As a novel approach, we suggest that ubiquitin pathway enzymes, in particular E3 ligases, are attractive therapeutic targets for the treatment of neurodegenerative diseases including AD. The best understood role of the RING finger E3 ligase, IDOL (Inducible Degrader Of the LDLR) is the regulation of LDLR stability and serum cholesterol levels and as such IDOL is a promising target for hypercholesterolemia. IDOL also promotes degradation of two major receptors for the secreted glycoprotein Reelin in hippocampal neurons. Reelin plays a critical role in memory and cognition; its binding to ApoER2 or VLDLR induces a series of signaling events culminating in increased long term potentiation (LTP) and synaptic plasticity. Mutant mice that lack Reelin (reeler) or ApoER2 and VLDLR exhibit a similar neurological phenotype and elevated tau phosphorylation, associated with neurodegeneration. Furthermore, mice deficient in ApoER2 and VLDLR have defects in LTP and memory. More important, Reelin enhances learning and memory in mouse models. Thus, enhancing Reelin signaling should be beneficial in treating cognitive decline in AD. Here we report the discovery and characterization of novel IDOL inhibitors that modulate LDLR as well as Reelin receptors.

These molecules bind directly to IDOL and may perturb IDOL:Substrate interactions. The most promising compounds are being studied in cellular and translational models. Data will be presented summarizing our progress in targeting IDOL for the treatment of neurodegenerative diseases.

Disclosures: **S. Kumar:** A. Employment/Salary (full or part-time):; Progenra Inc.. **T.R. Butt:** None. **M. Eddins:** A. Employment/Salary (full or part-time):; Progenra Inc. **J. Wu:** A. Employment/Salary (full or part-time):; Progenra Inc. **J. LaRocque:** A. Employment/Salary (full or part-time):; Progenra Inc. **S. Agarwal:** A. Employment/Salary (full or part-time):; Progenra Inc. **J. Marblestone:** A. Employment/Salary (full or part-time):; Progenra Inc. **M. Kodrasov:** A. Employment/Salary (full or part-time):; Progenra Inc. **B. Nicholson:** A. Employment/Salary (full or part-time):; Progenra Inc..

Poster

408. Alzheimer's Disease: APOE and Cholesterol

Location: Halls A-C

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Program#/Poster#: 408.11/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 1R03AG043784

Title: Presenilin mutations influence processing and trafficking of the ApoE receptor apoEr2

Authors: W. WANG[1], *S. W. BARGER[2,3]
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Abstract: The apoER2 receptor is a member of a family of cell-surface lipoprotein receptors which have been associated with Alzheimer's disease (AD) by genetics, biochemistry, and neuropathology. An important link of apoER2 to AD is conferred by apolipoprotein E (ApoE), which binds apoER2 alone or as a component of lipoprotein complexes; polymorphisms in the ApoE gene are responsible for the most prevalent genetic risk factor for late-onset AD. Presenilin (PS)-1 also has a genetic impact on AD, with mutations in its gene (PSEN1) causing early-onset AD with a high-penetrance, autosomal-dominant inheritance. Presenilins contribute to the γ-secretase component of amyloid β-peptide (Aβ) production, and certain mutations in PSEN1 appear to alter this proteolysis. However, PS1 also influences other aspects of cellular

physiology, including calcium regulation and trafficking of proteins through the secretory pathway. ApoER2 has been shown to be a substrate of γ-secretase. We have begun to examine the influence of PSEN1 mutation on levels, processing, and trafficking of apoER2. In mouse embryonic fibroblasts (MEF) carrying the PS1 mutation R278I, we find impaired γ-secretase activity for apoER2 in the basal state or after ApoE binding. Other effects of PS1 mutation were unmasked by attenuating γ-secretase activity with the inhibitor DAPT. PSEN1/PSEN2-/- MEF were transfected with PS1 of wild-type sequence or the L282V mutation; in the presence of DAPT, more apoER2 C-terminal fragment (CTF) accumulated the L282V-expressing cells compared to WT transfectants. The combination of DAPT and the L282V mutant also prevented the loss of apoER2 from the cell surface stimulated by ligand. These findings suggest that mutations of PS1 that cause AD alter the processing and perhaps the trafficking of apoER2.

Disclosures: W. Wang: None. **S.W. Barger:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Program#/Poster#: 408.12/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS37853

Alzheimer Association Zenith Award (C.I.)

AHA 09SDG2060701

Title: The apolipoprotein E4 (apoE-ε4) allele is associated with neurovascular dysfunction and predisposes to white matter injury and cognitive impairment in mice

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Abstract: The apolipoprotein E4 (apoE-ε4, apoE4) allele is a risk factor for microhemorrhages and white matter ischemic lesions (Neurology 81:292, 2013). However, the factors underlying the increased cerebrovascular risk remain to be defined. We tested the hypothesis that the apoE4 genotype is associated with alterations in the regulation of the cerebral circulation and increased white matter susceptibility to ischemic injury. To this end, we used human apoE targeted

replacement mice (apoE3 and apoE4) (Sullivan et al., JBC, 272:17972, 1997) (3-4 months; n=5/group) in which cerebral blood flow (CBF) was measured by laser-Doppler flowmetry in the somatosensory cortex under anesthesia. In apoE4 mice, the increase in CBF produced by whisker stimulation (WS) or by topical application of the endothelium-dependent vasodilator acetylcholine (ACh) was attenuated (WS, -49±3%; ACh, -38±3%; $p<0.05$). Next, we investigated if the neurovascular dysfunction increased the propensity of apoE4 mice to develop white matter ischemic lesions. White matter ischemia was induced in the corpus callosum by bilateral carotid artery stenosis (BCAS) with microcoils (0.18mm) for 4 weeks. BCAS led to more severe white matter injury in apoE4 than in apoE3 mice. Thus, BCAS reduced myelin density more in apoE4 (FluoroMyelin relative fluorescence units: 10±1) than in apoE3 mice (15±1), a reduction of 33% ($p<0.05$). Double immunolabeling with the voltage gated Na channel Nav1.6 and the paranodal contact protein Caspr revealed increased disorganization of the axonal nodal structure in apoE4 mice, assessed by the NaV1.6/caspr index (normal index 0.5; BCAS apoE3: 0.58±0.13; BCAS apoE4: 1.05±0.13; $p<0.05$ from apoE3). BCAS induced impairments in spatial memory, assessed by the arm alternation rate (%) in the Y-maze test, an effect worse in apoE4 (43±3%) than apoE3 (52±3) ($p<0.05$). These findings suggest that the apoE4 genotype is associated with cerebrovascular dysfunction, which leads to more severe white matter disruption and cognitive deficits following exposure to chronic cerebral hypoperfusion. Cerebrovascular alterations in apoE4 carriers are likely to play a role in the cognitive impairment associated with vascular risk factors and in the ischemic pathology associated with Alzheimer's disease.
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Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 408.13/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG041971

Title: Influence of ApoE on LRP1 function

Authors: *B. SHACKLETON, F. CRAWFORD, C. BACHMEIER
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Abstract: Alzheimer's disease (AD) is a neurodegenerative process characterized, in part, by accumulation of the beta-amyloid (Aβ) protein in the brain and cerebrovasculature. Mounting evidence suggests the excessive accumulation of Aβ in the AD brain is not due to aberrant Aβ production, but the result of impaired Aβ clearance mechanisms. One explanation for the attenuated clearance in AD is dysfunctional Aβ transport at the blood-brain barrier (BBB). The BBB transporter primarily responsible for the brain-to-blood elimination of Aβ is the low density lipoprotein receptor-related protein 1 (LRP1). While LRP1 interacts with an array of ligands, one of the more closely associated is apolipoprotein E (apoE). To determine the role of apoE in the regulation of LRP1 transport, we examined the effects of apoE on the proteolytic shedding of LRP1 to its non-functional soluble form. We identified apoE isoform-specific differences in LRP1 shedding *in vitro* and *in vivo*. Furthermore, LRP1 shedding showed a strong inverse correlation with the clearance of Aβ from the brain to the periphery. On further investigation, we identified the involvement of MMP9 and the α-secretase ADAM10 in the proteolysis of LRP1. Activity assays with these sheddases demonstrated apoE isoform specific modulations of their activity; with an inhibitory action demonstrated by MMP9 (apoE2>apoE3>apoE4) and an increase in activity with ADAM10 (apoE2>apoE3>apoE4). Our results indicate an inverse relationship between LRP1 shedding and Aβ transit across the BBB that is apoE isoform-specific. Moreover, we observed an interaction between apoE and the enzymes responsible for LRP1 proteolysis, which may explain the differential effects of the apoE isoforms in removing Aβ from the brain and offer new strategies for the treatment of AD.

Disclosures: B. Shackleton: None. **F. Crawford:** None. **C. Bachmeier:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS074536

Title: High-throughput screening for small molecule modulators of ApoE in primary human astrocytes

Authors: *G. M. FINAN[1], R. B. REALUBIT[2], C. KARAN[2], T.-W. KIM[1]
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Abstract: Apolipoprotein E (apoE) plays a key role in cholesterol transport in the brain. The ε4 allele of the gene encoding apoE (APOE) underlies the single most prominent risk factor for late-onset AD. Abundant evidence indicates that different apoE isoforms (E2, E3 or E4) differentially affect the clearance and accumulation of amyloid β-peptide (Aβ), as well as neuroinflammation and neuronal functions in the brain. Small molecule-mediated enhancement of brain apoE levels, especially regarding the E2 or E3 apoE isoforms, has been suggested as a promising therapeutic strategy. ApoE is mainly secreted from astrocytes in the brain, and certain agents that promote apoE secretion from astrocytes have been shown to potently reduce Aβ levels and reverse cognitive deficits in mouse models of AD. Nevertheless, molecular targets and pathways that mediate or modulate apoE secretion have not been systematically explored, particularly in primary CNS cells. In order to identify new chemical probes and potential therapeutic lead compounds based on apoE modulation in astrocytes, we performed 384-well plate high throughput screening (HTS) using immunological detection of secreted apoE in primary human astrocytes. Using this assay, we screened libraries consisting of known drugs and bioactive compounds as well as structurally-diverse scaffolds to identify new apoE-enhancing compounds. The identified hit compounds include small molecule modulators of cholesterol transport and synthesis, including neurotransmitter receptors and kinases. Our studies have a potential to identify new tool compounds that modulate apoE secretion through previously unknown mechanisms of action, or structurally novel compounds that are ideal for further AD drug discovery.

Disclosures: G.M. Finan: None. **R.B. Realubit:** None. **C. Karan:** None. **T. Kim:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Program#/Poster#: 408.15/H12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P01 AG030128-01

NIH NINDS 5T32NS041218

Title: APOE-associated biomarkers and their modulation by ibuprofen *in vivo*

Authors: *A. M. DIBATTISTA[1], S. B. DUMANIS[1,2], M. J. LADU[3], G. W. REBECK[1]
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Abstract: There is a growing body of evidence indicating that Alzheimer's disease (AD) risk factor apolipoprotein E (APOE) impairs brain processes before disease onset. While APOE research has focused on its effects on the pathological processes of AD, the role of APOE in the normal brain is not as well understood. Here, we characterize behavioral and biochemical differences accompanying the APOE risk genotype in an animal model without gross AD pathology (APOE Targeted Replacement (TR) mice). We previously published that APOE4 TR mice demonstrated impaired spatial memory and reduced dendritic spine density compared to APOE3 TR mice. In the present study, we report that APOE genotype alters levels and distribution of apoE-related proteins in the brains of APOE4 TR mice. APOE4 TR mice had an altered distribution of apoE protein between the TBS and TBS-X soluble brain fractions in both the hippocampus and cortex. Moreover, APOE4 TR mice had higher levels of ABCA1 and ABCG1 mRNA, and we confirmed a threefold higher level of ABCA1 in APOE4 TR brains through western blot analysis of brain extracts. Epidemiological studies have also shown non-steroidal anti-inflammatory drugs can reduce AD risk specifically in APOE4 carriers, and we tested whether treatment of mice with ibuprofen affected these various biomarkers. Two months of ibuprofen shifted the distribution of apoE and ABCA1 levels toward what we observed in APOE3 TR mice. Further, ibuprofen rescued spatial learning and memory deficits in APOE4 TR mice during the Barnes maze task in which mice are trained to locate an escape hole. While untreated APOE4 TR mice learn the task at a significantly slower rate, ibuprofen treated APOE4 TR mice learn the task at the same rate as APOE3 TR mice during training days. Similarly, while untreated APOE4 TR mice take significantly more time to locate the original location of the escape hole upon its removal, ibuprofen rescues this effect 24 hours, but not 72 hours, following training. We are currently investigating whether biochemical biomarkers can be modulated in APOE3 TR mice, and whether we can use other targeted drug treatments to induce similar effects.

Disclosures: A.M. Dibattista: None. **S.B. Dumanis:** None. **M.J. LaDu:** None. **G.W. Rebeck:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Program#/Poster#: 408.16/I1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Midwestern University

Arizona Alzheimer's Consortium

Title: Young adult carriers of the Alzheimers disease risk factor APOE4 show broad dysregulation in cortical energy metabolism pathways

Authors: *J. VALLA[1,4], M. PERKINS[1,4], D. SHONEBARGER[2], P. PANGLE[2], L. BALLINA[2], J. VALLEJO[3,4], G. JENTARRA[1,4]
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Abstract: The apolipoprotein E ε4 (APOE4) allele dramatically increases risk for development of late-onset sporadic Alzheimer's disease (AD). Imaging studies using FDG PET showed that young-adult APOE4 carriers display a regional pattern of reduced cerebral glucose metabolism similar to that in AD patients. Similarly, we have previously demonstrated that young-adult (age 18-40y) APOE4 carriers show postmortem functional declines in cortical oxidative metabolism in a laminar pattern closely resembling that of AD patients. These functional changes were apparent in the absence of any significant changes in AD-related neuropathology such as amyloid or tau deposition. Thus, to determine the foundation of these changes, we analyzed select targets in glucose uptake and metabolism, ketone uptake and metabolism, and mitochondrial oxidative metabolism in a subset of these subjects via SDS-PAGE, Western blotting and qPCR. Subjects consisted of N=12 APOE4 carriers and N=12 APOE4 noncarriers, 18-40 years of age. Frozen posterior cingulate/precuneus cortex samples received from the NICHD Brain and Tissue Bank for Developmental Disorders (University of Maryland, Baltimore) were processed for Western blotting and RNA extraction. Analysis of protein levels demonstrated statistically significant ($p<0.05$) increases in neuronal glucose transport (GLUT3) and phosphorylation (HEX1), neuronal monocarboxylate (ketone/lactate) transport (MCT2) and ketone catabolism (succinyl CoA transferase [SCOT]), and the apparent expression of select subunits of mitochondrial oxidative phosphorylation (Complexes I-V). Several of these changes have been further validated via qPCR. In contrast, the primary blood-brain barrier monocarboxylate transporter (MCT1) showed significantly lower protein expression in the APOE4 carriers. GLUT1 and caveolin-1 (CAV1) as well as the metabolic transcriptional co-regulator PGC-1α showed no expression differences between groups. These findings confirm that brain bioenergetic dysregulation may contribute significantly to the risk APOE4 confers for future AD, although it is not yet known what mechanism confers this vulnerability. One potential candidate is the decrease of brain ketone transport and subsequent compensation for the loss of this important brain fuel, which may have connotations for recent attempts to ameliorate AD-related cognitive symptoms via ketogenic supplementation.

Disclosures: **J. Valla:** None. **M. Perkins:** None. **D. Shonebarger:** None. **P. Pangle:** None. **L. Ballina:** None. **J. Vallejo:** None. **G. Jentarra:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Alzheimer's Association NIRG

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Title: Effects of global deletion of ApoA1 and ApoE on amyloid pathology and cognitive decline in a mouse model

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Abstract: Apolipoprotein A1 and E (ApoA and ApoE) combine with lipids in the body to form molecules called lipoproteins, which are responsible for packaging and transport of cholesterol and other fats. The most important apolipoproteins in brain cholesterol homeostasis are ApoE, synthesized and secreted by astrocytes, and ApoA-I, derived from plasma. ApoA-I is also the most abundant apolipoprotein in the periphery and the major protein component of HDL in plasma. We previously shown that the lack of ApoA-I aggravates memory deficits in APP/PS1ΔE9 mice in parallel to significantly increased cerebral amyloid angiopathy (CAA). The overexpression of human ApoA-I in a mouse model of Alzheimer disease, however, preserves cognitive function and attenuates neuroinflammation and CAA. Furthermore, it has been demonstrated that the lack of endogenous ApoE increases the clearance of Aβ from the

brain and dramatically reduces amyloid plaque pathology. In regard to AD-like pathology, in AD models, reduced levels of both apoA-I-I and apoE of Abca1ko mice are poorly understood.Here we generated APP/PS1 transgenic mice with global deletion of ApoE and ApoA-I (APP/PS1/DKO). We are comparing the amyloid pathology and cognitive decline of APP/PS1/DKO to those of APP/PS1/Abca1ko and APP/PS1/wt at 6 and 12 months of age.. Our results shown that APP/PS1/ABCA1ko mice demonstrated the highest level of cognitive impairment, followed by APP/PS1/ApoA-Iko. We examined amyloid pathology by measuring soluble and insoluble Aβ levels in these mice by ELISA and IHC. Our experiments again show that the APP/ABCA1ko mice have highest level and APP/PS1/DKO displaying the lowest level of amyloid plaques. Surprisingly, APP/PS1/DKO and APP/PS1/ApoEko single knockout mice had very little soluble Aβ suggesting that in these mice its efflux from brain is increased. We also examined Aβ clearance through *in vivo* microdialysis and radioactive labeled Aβ clearance studies. We conclude that the complete absence of brain apolipoproteins E and A-I increases the clearance of Aβ from the brain and diminishes the overall amyloid pathology.

Disclosures: **N.F. fitz:** None. **A.Y. Carter:** None. **E.L. Castranio:** None. **I. Lefterov:** None. **R. Koldamova:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Program#/Poster#: 408.18/I3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH T32 NS041218

NIH R01 AG035379-04

NSF POLS 1205919

Title: Effects of LXR activation on dynamics within *in vitro* networks of neurons using a multielectrode array (MEA) system

Authors: *G. A. RODRIGUEZ[1,2], X. CHEN[4,3], G. W. REBECK[1,2], R. DZAKPASU[4,3,2]
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Abstract: LXR agonists have received considerable attention in the recent literature for their ability to ameliorate cognitive deficits and reduce Aβ levels in the brains of transgenic AD mice. However, basic research investigating the influence of LXR activation on neuronal network signaling is lacking, and yet necessary to further elucidate mechanisms that underlie LXR agonists' therapeutic effects *in vivo*. In these studies, we address the hypothesis that LXR activation can facilitate network activity by impacting bursting dynamics within primary rat hippocampal neurons cultured on multielectrode (MEA) arrays. The MEA contains 59 titanium nitride electrodes arranged on an 8x8 grid capable of recording extracellular action potentials from a distributed network of neurons. The ability to record non-invasively from the networks over time makes this an ideal *in vitro* system for pharmacological studies. Primary hippocampal cells from E18 Sprague Dawley rats were treated with 1μM or 5μM TO-901317 for 120hr (DIV14-19). Media containing the drug was then exchanged for fresh media and the cells were maintained until DIV25. Dimethyl sulfoxide (DMSO) treated cultures served as vehicle controls. Parallel immunocytochemistry experiments showed ample astrocyte cell populations mixed with neurons. Western blot analyses showed activation of LXR due to chronic TO-901317, as secreted ApoE levels were increased in media from drug treated MEAs at DIV19 and 25. Spontaneous network activity was recorded for 15 min each day (DIV14-25). Overall, we found no difference in total number of spikes between treatment groups or number of spikes not in bursts. However, we discovered a modest effect of LXR-activation on bursting dynamics. While the number of bursts increased in untreated as well as DMSO-treated cultures over time (DIV17-25), hippocampal cells treated with TO did not exhibit similar increases in the burst counts over the recording sessions. Interestingly, burst duration appeared to increase in TO-treated cultures despite the decrease in the number of bursts, which might be accounted for by larger inter-spike intervals within bursts rather than an increased number of spikes within bursts. The effects of TO-901317 include an increased expression of genes encoding proteins involved in cholesterol metabolism, including ApoE and ATP-binding cassette transporters (ABCA1 and ABCG1). We suggest that these increases might facilitate network dynamics after modulations in excitability, such as synaptic potentiation. To this end, we will perform additional studies investigating the effects of LXR-activation in hippocampal cultures subjected to chemical Long-Term Potentiation.

Disclosures: G.A. Rodriguez: None. **X. Chen:** None. **G.W. Rebeck:** None. **R. Dzakpasu:** None.

Poster

408. Alzheimer's Disease: APOE and Cholesterol

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KU startup research funds

Alzheimer's Association Grant IIRG-10-172459

Title: Human apolipoprotein E (APOE) isoforms differentially modulate glucose and amyloid metabolic pathways in female brain: Implications for Alzheimer's disease prevention and early but not late intervention

Authors: *J. T. KEENEY, L. ZHAO
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Abstract: Three major genetic isoforms of apolipoprotein E (APOE) exist in humans, APOE2, APOE3, and APOE4, leading to differences in susceptibility to Alzheimer's disease (AD). The most common genotype, APOE3, believed to be neutral in AD, is present in approximately 75% of the population. APOE4, potentially the strongest genetic risk factor for AD, occurs in only 20% of people but accounts for approximately 65% of AD cases. The third, APOE2, believed to be a protective variant against AD, is rare, present in 5% of the population. The purpose of this study was to elucidate differences in key mechanistic pathways in preclinical AD that arise from genetic differences in APOE isoforms and to identify potential targets for AD prevention and early intervention. Hippocampal RNA samples collected from middle-aged female mice with targeted human APOE2, APOE3, and APOE4 gene replacement were analyzed with a qRT-PCR custom array for the expression of a focused panel of genes involved in insulin/IGF signaling. APOE2 brain exhibits the most metabolically robust profile among the three APOE brains. When compared to APOE2 brain, APOE3 and APOE4 brains exhibit markedly reduced levels of insulin-like growth factor1 (IGF1), insulin receptor substrates (IRSs), and facilitated glucose transporter 4 (GLUT4). Additionally, APOE4 brain exhibits further reduction in insulin-degrading enzyme (IDE), a major enzyme that degrades amyloid beta (Aβ) peptide monomers in the brain as well as being involved in promoting glucose metabolism. This data provides the first documented evidence that human APOE isoforms differentially affect the IGF1/GLUT4/IDE system in the brain. Downregulation of IGF1, GLUT4, and IDE associated with APOE4 could lead to detrimental changes in downstream glucose and amyloid metabolism, both of which could further contribute to increased AD risk in APOE4 carriers. Supporting evidence is provided by protein level and post-translational modification assessment and functional outcomes of mitochondrial respiration and ATP production using Oxygraph high-resolution respirometry. IGF1 polymorphism has been associated with increased risk for AD. Elevation of monomeric Aβ and glucose hypometabolism have been shown as accentuated changes occurring in preclinical AD brains. Therefore, these data suggest that a therapeutic strategy that enhances brain IGF1/GLUT4/IDE signaling activity holds promise for preventing, reducing the risk of

developing or, at least, delaying the onset of AD, particularly in the high-risk APOE4 carriers, as well as for AD early intervention especially when addressed in the preclinical stages.

Disclosures: J.T. Keeney: None. **L. Zhao:** None.

Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

Support: CONACYT CB-167821-2011

CONACYT 265189

FAI2011 (C12-FAI-0362.62

PROMEP/103.5/10/7697

Title: Long term tolerance to human neural mesencephalic precursor transplant in a parkinson´s disease model

Authors: *C. A. SALAZAR-ALDRETE[1], H. GONZALEZ-SANCHEZ[1], V. RODRIGUEZ[2], C. CASTILLO[3]
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Abstract: Striatal transplants of neural midbrain precursor cells improve drug-induced rotation in hemiparkinsonian rats (Courtois et al 2010). However, side effects caused by use of immunosuppressive drugs cut off behavioral test at three month after transplantation. This trouble limited the evaluation of maturity and integration of host tissue transplant to a short period of time. In the present study, we developed a long term tolerant model induced by early post-natal exposure to cells. METHODS. Wistar rats were randomly distributed in three groups (n= 8 per group): Group 1, rats that received an oral dose of 100 000 cel/μL (hVM1) on postnatal days 1-5 + Transplant of dopaminergic cells; Group 2, rats that received an oral dose of 100 000 cel/μL (hVM1) on postnatal days 1-5 but without transplant and Group 3, rats without tolerance treatment. Rats received a 6-OHDA injection in right medial forebrain bundle. Four week after

lesion, then movement asymmetry was corroborated by injection only rats exhibiting at least 4 contralateral rotation/min in response to apomorphine (0.25 mg/kg) were selected for further striatal transplantation studies (AP + 0.5,ML -3.3,DV -4.5). RESULTS: Animals of group 2 or group 3 showed an increasing of turning behavior while the tolerant model group displayed an improvement in drug-induced rotation evaluated after six months to tolerance induction. Our results are pointing to transplantation of dopamine-producing cells can produce long-term alleviation of behavioral turning asymmetry in an oral tolerant animal model able to generate changes enough to maintain the survival of the transplant into host without immunosuppression; however, cell engraftment and integration of transplanted cells remain to be elucidated.

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Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson’s Disease

Support: CNPq

CAPES

FAPESP

FAPESPA

Title: An *in vitro* experimental model of Parkinson’s disease: Effects of rotenone, neuroprotection by antioxidants and mechanisms of cell death

Authors: *A. J. FILHO[1,2], D. C. F. LOPES[3], C. S. MATSUBARA[3], M. N. SILVA[4], E. S. YAMADA[4], C. SCAVONE[3], C. D. MUNHOZ[3], E. T. COSTA[4]
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Abstract: Despite different possible causes of Parkinson’s disease (PD), many studies have suggested important roles for mitochondrial impairment, oxidative processes as well as environmental factors in the pathogenesis of Parkinson's disease. In this context, one model to

study PD is the administration of the plant-derived pesticide rotenone which is a specific inhibitor of mitochondrial complex I, inducing apoptosis by generating mitochondrial reactive oxygen species and originating effects in rats which closely resemble PD. Therapeutic efforts aimed at removal of free radicals or prevention of their formation may be beneficial in PD. Regarding this point, natural products with antioxidant properties are an attractive source of potential cytoprotective compounds. The purpose of this study was: (1) to investigate the neuronal death induced by rotenone using mixed neuron/glia primary cultures from hippocampal and ventral mesencephalon of Wistar rats (*Rattus norvegicus*), (2) to investigate the role of calcium ions in this experimental model and (3) to investigate the potential cytoprotective effects of antioxidant-rich aqueous extract from mahogany leaves (*Swietenia macrophylla*). Neuronal death was analyzed with colorimetric assays for the production of MTT and lactate dehydrogenase enzyme activity (LDH). Our results showed significant concentration-dependent reduction in cell viability after exposure to rotenone both in cultures derived from hippocampus and ventral mesencephalon. We also demonstrate a discreet role of mitochondrial calcium in this rotenone-induced cell loss. Moreover, aqueous extract from mahogany leaves at non-toxic concentrations was not cytoprotective in this model. We also found that rotenone induced cell death by necrosis and apoptosis at the concentrations tested, as analyzed by MTT and LDH assays, as well as by Western blot analysis for specific necrosis and apoptosis markers. The results of this study should advance our knowledge on the mechanisms of action of environmental factors in the pathogenesis of Parkinson's disease. Further studies are still needed to elucidate the participation of calcium homeostasis in the mechanisms of cell death, as well as the use of antioxidant compounds as cytoprotective therapies in PD.

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Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson’s Disease

Title: Longitudinal PET assessment of metabolic changes associated with slow dopaminergic depletion in the MPTP primate model of Parkinson Disease

Authors: *F. MOLINET-DRONDA[1,2,3], J. BLESA[1,4], C. JURI[5], M. COLLANTES[2,6], E. IGLESIAS[1,3], I. PEÑUELAS[2,6], J. A. OBESO[1,3,7]
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Abstract: Parkinson´s disease (PD) is mainly characterized by progressive loss of dopaminergic neurons in the substantia nigra causing dopamine depletion in the striatum. This triggers a succession of compensatory changes delaying the appearance of motor manifestations, leading to a prolonged pre-motor period of disease evolution. Here, we describe the metabolic pattern associated with progressive nigro-striatal lesion in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model. **Aim**: To define the metabolic changes associated with different states of striatal dopamine depletion. **Methods**: Twenty-four monkeys (*Macaca fascicularis*) were administrated MPTP using 0.5 mg/kg weekly to obtain different groups: *Baseline*, *asymptomatic*, *recovered, mild,* and *severe* parkinsonian, according to Kurlan scale assesments. An additional group (n=6) was studied at *baseline*, *severe* parkinsonian and after *L-Dopa* treatment for 14 weeks. PET imaging was performed using monoaminergic (^{11}C-dihydrotetrabenazine; ^{11}C-DTBZ) and metabolic (^{18}F-fluorodeoxyglucose; ^{18}F-FDG) radiotracers. Regions of interest (ROI) analysis were done for ^{11}C-DTBZ PET (striatum) and Statistical Parametric Mapping (SPM) analysis for ^{18}F-FDG studies (whole brain). **Results**: ^{11}C-DTBZ PET images showed a progressive decrease of Binding Potential (BP) values in the striatum throughout MPTP administration and the ensuing of parkinsonian signs. ^{18}F-FDG PET in the parkinsonian state was characterized by reductions in posterior parietal, temporal lobe, lateral premotor and frontal cortex, as well as increases in the thalamus, globus palidus and cerebellum. ^{18}F-FDG PET after L-Dopa administration revealed a metabolic pattern recognized by hypometabolism in posterior parietal-occipital region and hipermetabolism in post-comisural putamen, thalamus and midbrain. **Conclusions**: Progressive dopaminergic striatal depletion in this MPTP monkey model led to recognizable parkinsonian metabolic patterns that are homologous to the clinical changes observed on patients. Moreover, we report the first demonstration of metabolic brain networks at different DA depletion stages in a nonhuman primate model of parkinsonism. Supported by CIBERNED, UTE-FIMA.

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Poster

409. Parkinson's Disease Models II

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Program#/Poster#: 409.04/I8

Topic: C.03. Parkinson's Disease

Support: German Research Foundation, Clinical Research Group (KFO 219)

Title: Mesolimbic system in Parkinson's disease: Degeneration of the medial forebrain bundle

Authors: S. SRITHARAN[1,2], E. A. PELZER[1], C. MELZER[1], *R. GRAF[1], L. TIMMERMANN[2], M. TITTGEMEYER[1]
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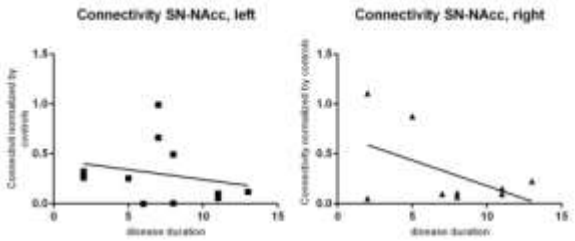
Abstract: It is becoming increasingly clear that non-motor systems play a major role in Parkinson's disease. Many cognitive aspects such as learning, reward processing and memory are affected in PD and progress with disease duration. However, the underlying pathophysiology remains unclear. In this study we apply *in vivo* connectivity analysis following diffusion MRI and tractography to investigate pathological changes in an aspect of the medial forebrain bundle in PD connecting the substantia nigra and the nucleus accumbens. So far, 3 T diffusion and structural MRI images were acquired in 10 right-handed, non-demented male PD patients and 7 age-matched controls. All patients underwent L-Dopa-therapy. All MR-images were analysed with FSL 5.0.4 (http://fsl.fmrib.ox.ac.uk/fsl/). Masks of substantia nigra, caudate nucleus and nucleus accumbens 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. In our preliminary analysis, we find that: (1) diffusion MRI offers the possibility for an *in vivo* quantification of this aspect of the medial forebrain bundle; (2) PD patients show a degeneration in the connectivity between substantia nigra and the nucleus accumbens with disease duration. In conclusion we hypothesize, that degeneration in this part of the medial forebrain bundle may contribute to the evolution of psychiatric, non-motor symptoms in PD.



Disclosures: **S. Sritharan:** None. **E.A. Pelzer:** None. **C. Melzer:** None. **R. Graf:** None. **L. Timmermann:** None. **M. Tittgemeyer:** None.

Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

Support: NSFC of 30770746;81171061; 83171256;81361128012

Title: Characteristics of tremor related burst activity in the ventrolateral thalamus in patients with Parkinsonian rest tremor

Authors: ***P. ZHUANG**[1], M. HALLETT[2], T. LIU[1], Y. ZHANG[1], J. LI[1], Y. LI[1]
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Abstract: Objective: To characterize the tremor related oscillatory burst activity in the ventral oral posterior nucleus (the Vop, the pallidal relay nucleus) and the ventral intermediate nucleus (the Vim, the cerebellar relay nucleus) in patients with parkinsonian rest tremor. **Methods**: 26 PD patients with rest tremor who underwent thalamic surgery were studied. Microelectrode recordings in the Vop/Vim and EMG of contralateral limbs were performed during surgery. Single unit analysis and interspike interval (ISI) was used to assess neuronal mean firing rate (MSFR) and pattern. Bursts were detected by the "Surprise" method. The analysis includes: the temporal structure of peri-burst and burst segments; the pre-post burst length, the duration of burst and interburst intervals (IBI). The relationships between burst length, the duration of flanking ISIs and IBIs were performed. Power spectral analysis was performed to evaluate neuronal oscillation. Coherence analysis was used to study neuronal oscillatory activity in relation to limb EMG. Voluntary-responsive neurons and neurons located >3 mm anterior to the Vc border were presumed to be located in the Vop and kinesthetic-responsive neurons and those located <3 mm anterior to the Vc border were presumed to be located in Vim. **Results:** Of total 224 neurons identified, 68.3% (n=153) were oscillatory neurons. Of oscillatory neurons, 28.8% had β frequency (range of 8-30 Hz) and 71.2% (n=108) had tremor frequency (4-6 Hz) (TFB), of which, 91.7% (n=100) were coherent with limb tremor ($p<0.05$). The burst analysis showed that there were significant differences of burst characteristics between the Vop and Vim including: Intraburst rate (spikes/s): 108.4±29.2 vs 123.2±23.5; Burst duration: 41.4±9.3 ms vs 44.9±7.8

ms; Proportion of spikes in burst (%): 51.3±15.6 vs. 62.5±13.3; Proportion of time spent in burst (%):12.1± 6.0 vs 16.5± 5.3; IBI: 337.1±215.6 ms vs 203.1±67.8 ms. It was found that number spikes in burst significantly correlated with the length of pre- and post-burst ISI. However, no significant difference was reached between the Vop and Vim. Further analysis showed that the MSFR of oscillatory neurons in the Vop was lower than those neurons in the Vim (24.1±5.8 Hz vs 31.5±5.7 Hz, $p<0.05$). Similarly, the density of TFB oscillatory neurons in the Vop was significantly lower than those neurons in the Vim (0.31±0.29 /mm vs 0.68±0.7/mm). **Conclusion:** The data support the hypothesis that the altered interactions between the basal ganglia and the cerebellothalamic circuit seem to play an important role in resting tremor of PD.

Disclosures: P. Zhuang: None. **M. Hallett:** None. **T. Liu:** None. **Y. Zhang:** None. **J. Li:** None. **Y. Li:** None.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.06/I10

Topic: C.03. Parkinson's Disease

Title: iPSC based model systems to study Parkinson's Disease: Understanding the biology of PD and Drug screening

Authors: C. M. REVANKAR[1], B. J. HAMMER[1], K. BI[1], S. B. HERMANSON[1], D. V. THOMPSON[1], M. S. PIEKARCZYK[1], C. S. LEBAKKEN[1], L. J. REICHLING[1], T. SAMPSELL-BARRON[1], B. SCHUELE[2], J. LANGSTON[2], *D. R. PIPER[3,1], K. W. VOGEL[1]
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Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease in the world and affects 1-2% of the population over age 65. Pathologically, PD is marked by a loss of dopaminergic neurons in the substantia nigra pars compacta region of the brain. Because of lack of access to such tissue, or availability of good animal models of PD, iPSC-generated neurons hold promise in the development of model systems to study PD. Recently, biological links between LRRK2 mutation and PD have been reported. The most common mutant, G2019S, like some other LRRK2 mutations, has increased kinase activity, which correlates with increased neuronal cytotoxicity. We have generated iPSCs from patients harboring mutations in the PARKIN and LRRK2 genes, as well as a rare case with mutations in both the LRRK2 and GBA

genes, and a patient with Multiple Systems Atrophy (MSA)--a “Parkinson’s Plus Syndrome” disease with no known genetic determinants. To eliminate line-to-line variations due to genetic background, we have set out to generate a set of isogenic iPSC lines that differ at a single point in the genome using the Transcription Activator-Like (TAL) effector nuclease technology. For instance, we have deleted the -synuclein gene from the MSA line in order to understand the impact of Lewy bodies, and reverted the LRRK2 and GBA mutations back to wild type in order to better understand any synergies between these mutations. These iPSC lines have been differentiated to neural stem cells (NSCs), and further into dopaminergic neurons and glial cells. Using the NSCs, fluorescence-based, high-throughput compatible assays have been developed to monitor phenotypes that are associated with PD, such as oxidative stress, metabolic activity, apoptosis, mitochondrial function, and autophagy. The long-term goal is to use these optimized assays is to provide a platform that allows for the facile interrogation of small molecule compounds in “relieving” phenotypes associated with PD.

Disclosures: **C.M. Revankar:** A. Employment/Salary (full or part-time):; ThermoFisher. **B.J. Hammer:** A. Employment/Salary (full or part-time):; ThermoFisher. **K. Bi:** A. Employment/Salary (full or part-time):; ThermoFisher. **S.B. Hermanson:** A. Employment/Salary (full or part-time):; ThermoFisher. **D.V. Thompson:** A. Employment/Salary (full or part-time):; ThermoFisher. **M.S. Piekarczyk:** A. Employment/Salary (full or part-time):; ThermoFisher. **C.S. Lebakken:** A. Employment/Salary (full or part-time):; ThermoFisher. **L.J. Reichling:** A. Employment/Salary (full or part-time):; ThermoFisher. **T. Sampsell-Barron:** A. Employment/Salary (full or part-time):; ThermoFisher. **B. Schuele:** None. **J. Langston:** None. **D.R. Piper:** None. **K.W. Vogel:** A. Employment/Salary (full or part-time):; ThermoFisher.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.07/I11

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

Title: Intrastriatal alpha synuclein preformed fibrils in macaque monkeys: neuronal transport, long-term imaging and neuropathologic changes

Authors: *J. H. KORDOWER[1], Y. CHU[1], S. MULLER[1], A. TAVARES[2], O. BARRET[2], D. ALAGILLE[2], J. SEIBYL[2], G. TAMAGNAN[2], K. MAREK[2], K. C. LUK[3], J. Q. TROJANOWSKI[3], V. M. Y. LEE[3]
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Abstract: Misfolded alpha synuclein (α-syn) is an integral pathology in a variety of neurodegenerative disorders including Parkinson's disease. Recent observations in the human brain, coupled with preclinical studies, demonstrated the templated conversion of normal α-syn to pathological α-syn by α-syn preformed fibrils (PFFs). This, as well as the transport and transfer of templated pathological α-syn in rodent models, supports the concept that α-syn has prion-like properties and the transfer of this protein could be responsible for disease progression. We injected thrice sonicated human α-syn PFFs fibrils unilaterally into the putamen of 9 cynomolgus monkeys. One animal died unexpectedly 3 months post-injection. Compared to baseline, PE2I SPECT imaging in the remaining 8 monkeys showed a bilateral increase in dopamine transporter (DAT) in the striatum beginning 3 months post-surgery. This effect was seen on multiple scans for up to 12 months post-surgery. At 12 months the mean increase was 71% with a range of 17% to 120%. We sacrificed 4 of the 8 monkeys at 12 months. An occasional Lewy body was seen in the monkey that died 3 months post-injection. In the monkeys sacrificed one year post-injection, each showed robust expression of α-syn as well as ser129-phosphorylated α-syn pathology within the area of the injection site. There was also a robust conversion of normal α-syn to pathological α-syn and transport of pathological α-syn to the ipsilateral substantia nigra (SN). Thioflavin S positive Lewy bodies and neurites were also seen within the ipsilateral SN. Dopaminergic SN neurons containing ser129-phosphorylated pathological α-syn displayed approximately 50% less tyrosine hydroxylase optical density relative to neighboring neurons that were ser129-phosphorylated α-syn immuno-negative or normal nigral neurons seen in control-injected monkeys. Studies are underway to assess spread or transport beyond the nigrostriatal system. These data support the concept that α-syn PFFs convert monkey α-syn into pathological α-syn that spreads, presumably by axonal transport, in the primate brain. These data will also be discussed within the context of additional experiments performed in these monkeys as well as the remaining ones that will be sacrificed at 18 months post-surgery.

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Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

Support: Cure PD trust, UK

Title: Rescue of the pathological phenotype in α-synuclein(1-120) transgenic mice

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Abstract: Alpha synuclein is a 140- aa pre-synaptic protein associated to vesicles where it regulates release events by chaperoning the soluble NSF attachment protein receptor (SNARE) complex assembly and vesicle fusion (Nemani et al 2010; Burre' et al., 2010). Point mutations and duplication/triplications of α-synuclein cause familial forms of Parkinson's disease (PD), which is characterised by the aggregation of αsynuclein in the striatum in Lewy bodies (LB) (Ibanez et al., 2004; Spillantini et al., 1997, 1998). Part of the aggregated αsynuclein found in LBs of PD cases is C-terminally truncated and this post-translational modification increases the propensity of αsynuclein to aggregate and form toxic species although the mechanisms leading to αsynuclein aggregation are at present unclear (Baba et al., 1998; Tofaris et al., 2003; Burre' et al., 2012; Nakata et al., 2012; Janezic et al., 2013). Our previously described transgenic line containing C-terminally truncated αsynuclein, namely αsyn(1-120) is characterised by the presence of progressive formation of clumps of αsynuclein in the striatum, a re-distribution of SNARE proteins and defective dopamine release and by 12 months of age (Garcia-Reitbock et al., 2010). These features recapitulate several of the pathological hallmarks of early-onset PD and provide a valid model for the study of the molecular events that precede nigral degeneration Our hypothesis is that the presynaptic terminals are the initial site of neurodegeneration and therefore we focused on the study of the effect of key synaptic components on the ability to restore the synaptic deficit associated with α synuclein truncation. Here we show that the virally expressed co-chaperone of HSC70, CSPα not only rescues the DA release impairment but also reduces the pathophysiological "clumps" of αsynuclein in the striatum of αsyn(1-120) mice. Our results suggest that modulation of synaptic protein distribution represents a potential therapeutical target for restoring the deficit associated with the early stages of PD pathology thus slowing down or preventing nigral degeneration.

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Poster

409. Parkinson's Disease Models II

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Program#/Poster#: 409.09/J1

Topic: C.03. Parkinson's Disease

Support: KAKENHI 24591262

Title: Dopamine-mediated oxidation of methionine 127 in alpha-synuclein causes cytotoxicity and oligomerization of alpha-synuclein

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the selective loss of dopaminergic neurons and the presence of Lewy bodies. Many recent studies focused on the interaction between α-synuclein (α-syn) and dopamine in the pathogenesis of PD, and fluorescent anisotropy suggested that the C-terminal region of α-syn may be a target for modification by dopamine. However, it is not well understood why PD-related pathogenesis occurs selectively in dopaminergic neurons. We investigated the interaction between dopamine and α-syn with regard to cytotoxicity. A soluble oligomer was formed by co-incubating α-syn and dopamine *in vitro*. To clarify the effect of dopamine on α-syn in cells, we generated PC12 cells expressing human α-syn, as well as the α-syn mutants, M116A, Y125D, M127A, S129A, and M116A/M127A, in a tetracycline-inducible manner (PC12-TetOFF-α-syn). Overexpression of wildtype α-syn in catecholaminergic PC12 cells decreased cell viability in long-term cultures, while a competitive inhibitor of tyrosine hydroxylase blocked this vulnerability, suggesting that α-syn-related cytotoxicity is associated with dopamine metabolism. The vulnerabilities of all mutant cell lines were lower than that of wildtype α-syn-expressing cells. Moreover, α-syn containing dopamine-mediated oxidized methionine (Met(O)) was detected in PC12-TetOFF-α-syn. Met(O) was lower in methionine mutant cells, especially in the M127A or M116A/M127A mutants, but also in the Y125D and S129A mutants. Co-incubation of dopamine and the 125YEMPS129 peptide enhanced the production of H2O2, which may oxidize methionine residues and convert them to Met(O). Y125- or S129-lacking peptides did not enhance the dopamine-related production of H2O2. Our results suggest that M127 is the major target for oxidative modification by dopamine, and that Y125 and S129 may act as enhancers of this modification. These results may describe a mechanism of dopaminergic neuron-specific toxicity of α-syn in the pathogenesis of PD.

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Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

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CONACYT Fellow at EFG 255122

Posgrado en Ciencias Biológicas, UNAM

Gabino Borgonio Pérez

Title: Effect of oxidative stress on the glutathione system and the neuromelanin formation in substantia nigra of rats exposed to low doses of ozone

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Abstract: Oxidative stress is involved in various intracellular signaling. When the antioxidant species are insufficient to maintain the redox balance, a state of oxidative stress occurs which causes damage and loss of cellular functions, as seen in neurodegenerative disease such as Parkinson's disease (PD). Moreover the formation of neuromelanin, depend on the REDOX equilibrium during oxidative metabolism of dopamine. In PD, oxidized dopamine interacts cysteine wich is a precursor of glutathione. The aim of this work was to study the effect of oxidative stress on the relationship between the glutathione system and the formation of neuromelanin in the substantia nigra (SN) of rats chronically exposed to low ozone doses (O3). Method: sixty male Wistar rats with free access to food and water were randomly divided into the following 6 groups (n=10): group 1) control; group 2) O3 7 days; group 3) O3 15 days; group 4) O3 30 days; group 5) O3 60 days and group 6) O3 90 days. Ozone was daily administered for 4 h to 0.25 ppm. At the end of the treatment, the animals were anesthetized with sodium pentobarbital (50 mg/Kg) and each group was divided into 2 subgroups. In the first subgroup (n=6) animals were perfused and their brains were processed with histochemical techniques for neuromelanin (N). The second subgroup (n=4) rats were decapitated for obtaining fresh tissue of

SN and were processed with spectrophotometric techniques to measure the activity of glutathione peroxidase (GPX), glutathione reductase (GR), total glutathione (GT), glutathione reduced (GSH) and oxidized glutathione (GSSG). The results show an increase in GPX activity at 7 and 60 d and a decrease at 30 and 90 d ($p < 0.05$). The GR has a significant increase from 7 to 90 d compared to the control group. The GSSG presents a significant increase at 7, 15 and 60 d and a reduction at 30 and 90 d ($p<0.05$) compared to the control group. GSH showed a significant increase at 30 d and a decrease at 60 and 90 d ($p<0.05$) against control group. The neuromelanin shows a significant increase at 7 and 15 d and decreased at 60 and 90 d compared to the control group ($p < 0.05$). In conclusion exposure to O3 causes glutathione system alteration and block neuromelanine formation, which depends on the time of exposure to this gas. Therefore, oxidative stress caused by ozone exposure, contributes to the process of the progressive neurodegeneration in SN similar to what may be happening in PD.

Disclosures: E.D. Ferreira: None. **S. Rivas-Arancibia:** None.

Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson’s Disease

Support: National Natural Science Foundation of China (91332206)

Title: Characterization of Parkin expression in aged rhesus monkeys

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Abstract: Parkinson’s disease (PD) is an age-dependent neurodegenerative disorder that can be caused by genetic mutations. The loss of function of parkin caused by parkin gene mutations has been found to be associated with autosomal recessive early onset PD. Studies have suggested that parkin is capable of degrading the toxic forms of α-synuclein via its E3 ubiquitin ligase activity that targets proteins for proteasomal degradation, resulting in protecting neuronal cells from oxidative stressors. It remains to be investigated how parkin is expressed in the primate brain regions, especially in the aged substantia nigra that is particularly affected in PD. Here we examined the expression of parkin in the brain and peripheral tissues of rhesus macaque at 2, 8,

and 22 years of age. Western blot analysis showed that there are mainly two isoforms of parkin protein (52 kD and 45 kD) expressed in monkey tissues. While the expression of parkin is relatively stable in peripheral tissues at different ages, the level of parkin is markedly increased in the monkey brain regions from 2 to 8 years, suggesting that parkin function is important for neuronal function in the adult primate brain. Interestingly, there are varied expression levels of parkin in the substantia nigra in individual monkeys at 22 years of age. Our findings suggest that the different expression of parkin in the aged primate brain regions may contribute to the PD susceptibility during aging.

Disclosures: W. Yang: None. **Z. Tu:** None. **S. Li:** None. **X. Li:** None.

Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

Support: NIH/NIA 4R00AG033687

Title: Increased glutamate release in the dorsal striatum of a mitochondrial mouse model of parkinson's disease

Authors: *H. A. BOGER[1], A. FARRAND[1], R. GREGORY[2], K. HELKE[2]
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Abstract: Mitochondrial dysfunction has been implicated in the degeneration of dopamine (DA) neurons in Parkinson's disease (PD). In addition, many animal models of PD utilizing neurotoxins, such as 6-OHDA and MPTP, have shown that these toxins disrupt mitochondrial respiration by targeting complex I of the electron transport chain, thereby impairing DA neurons in these models. The MitoPark mouse model was created to mimic the mitochondrial dysfunction observed in the DA system of PD. These mice display the same phenotypic characteristics as PD including, accelerated decline in motor function and DAergic systems with age. In addition, these mice respond to L-dopa treatment and with age, they also develop L-dopa dyskinesia, however the mechanism is unknown. A potential cause of L-dopa dyskinesia is corticostriatal glutamate excitotoxicity. Therefore, the focus of this study was to determine glutamate neurotransmission in the dorsal striatum of 28 week old MitoPark mice. At this age, MitoPark mice are displaying significant motor deficits. Using *in vivo* electrochemical detection of

glutamate, MitoPark mice have greater KCl-stimulated release of glutamate compared to control mice. In addition, PCR analysis of dorsal striatal tissue indicate that MitoPark mice have greater RNA expression of glutamate-specific receptors, including mGluR3, mGluR5, and NMDAR-2A. This increased expression pattern coupled with increase release suggests that MitoPark mice have a greater risk for glutamate-induced toxicity, as it has been shown that continuous activation of post-synaptic glutamate receptors increases intracellular levels of Ca2+ and cell death. Studies are ongoing to correlate the changes in glutamate release to DAergic markers. Future studies will be conducted to determine if blocking glutamate receptors or increasing glutamate uptake will reduce/prevent L-dopa induced dyskinesia as associated with DAergic mitochondrial dysfunction.

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Poster

409. Parkinson's Disease Models II

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Program#/Poster#: 409.13/J5

Topic: C.03. Parkinson's Disease

Title: Dynamics of neurodegeneration in a slow progressive MPTP marmoset model for idiopathic Parkinson's disease

Authors: *S. TOLBOOM[1,2], R. E. VAN KESTEREN[1], S. O. HOFMAN[2], J. A. M. WUBBEN[2], A. B. SMIT[1], I. H. C. H. PHILIPPENS[2]
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Abstract: The current study investigates the dynamics and progression of neurodegeneration in a slow progressive model for idiopathic PD in marmoset monkeys (Callitrix jacchus). PD symptoms and pathology were elicited by intraperitoneal injections with a low dose of the neurotoxin MPTP on a weekly basis. Investigated were 1) the timing of symptom expression, 2) the individual variation of sensitivity towards MPTP, similar to the variation of disease severity in PD patients, and 3) the compensatory mechanisms after discontinuation of MPTP injections. Marmosets were selected from five different breeding families to obtain a genetically diverse group of animals for investigating familial aspects of PD. Animals were once weekly injected with MPTP (0.5 mg/kg, sc) and monitored over time using an extensive battery of behavioral assessments, including motor as well as non-motor related tests. In addition, we analyzed brain

tissue of animals from all disease stages using histology, to relate the disease symptoms to the pathological state, and with proteomics, which might reveal novel molecular targets in the development and progression of the disease. The slow induction of PD with low MPTP doses led to disturbances in both motor and non-motor-related behavior. As expected, motor-related behaviors were progressively impaired with disease progression. In addition, variation was observed in the onset and progression of parkinsonian symptoms, which correlated well with the animal's genetic background. These findings strongly suggest a genetic component, which will be further investigated using whole-genome sequencing. Strikingly, at the time of diagnosis, when only subtle parkinsonian symptoms were observed only mild degeneration was found in the substantia nigra. This suggests that degeneration starts in the axons of the affected neurons. Indeed, in the striatum a decline in levels of thyroxin hydroxylase, an enzyme necessary for the synthesis of dopamine, was found. In addition, all tested animals showed improvement of parkinsonian symptoms and motor related behavior after stopping MPTP treatment, which allowed us to uniquely address molecular and cellular mechanisms of compensatory processes. The slow progressive MPTP model for idiopathic PD provides a window of opportunity to test disease-modifying drugs on early progression and dynamics of PD. Moreover, this model allows us to study the recovery mechanism after MPTP discontinuation, leading to possible treatment options of PD. The integrative nature of the markers used and the human clinical validity of the marmoset model will contribute to a better understanding of the development and progression of the disease.

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Poster

409. Parkinson's Disease Models II

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Topic: C.03. Parkinson's Disease

Support: NS39006

the American Lebanese Syrian Associated Charities (ALSAC)

Title: Effect of aging on dopaminergic neurons, microglia and open field behavioral following mptp in c57bl/6j, swiss-webster and gstpi-null mice y. jiao1*, y. dou1, r. smeyne1 1developmental neurobiology, st. jude children's research hospital, memphis, usa

Authors: *Y. JIAO
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Abstract: Aging has been proposed as the major risk factor in the development of most neurodegenerative disorders, including Parkinson's disease (PD). Although it is likely that many systems are affected by aging, it is well recognized that during ageing there is a generalized increase in the oxidative stress state of the brain. Here, we examine the response of 3 strains of mice (C57BL/6, Swiss-Webster, GSTpi-/-) at three different ages (4 months, 12 months and 24 months) to an acute administration of a known inducer of oxidative stress, the parkinsonian toxin MPTP. We examined the SNpc for loss of SNpc DA neurons, induction of an inflammatory response by stereological assessment of resting and activated microglia as well as two measures of behavior: open field for general activity and rotorod for examination of motor coordination. We find that aging alone does not seem to have any significant effect on the number of SNpc DA neurons, astrocytes or microglia in C57B/6, Swiss-Webster or GSTpi-/- mice. Behaviorally, we observed that rotorod performance decreased as animal's age, but not difference was seen among genotypes; although there were noted differences between males and females. In open field, general activity was similar through 24 months in C57BL/6 and GSTpi-/- mice, but significantly increase in the Swiss-Webster. Exploratory behavior measured by rearing behavior was also stable in C57BL/6 and Swiss-Webster but declined in GSTpi-/- mice. Following MPTP, we find that as mice age, they do not increase their sensitivity to MPTP as measured by SNpc DA neuron number. In a similar manner ageing does not affect MPTP-induced changes in general activity.

Disclosures: Y. Jiao: None.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.15/J7

Topic: C.03. Parkinson's Disease

Title: Dendritic loss in the prefrontal cortex of MPTP-treated monkeys

Authors: *G. S. WALHA[1], R. F. MERVIS[2,3], S. K. FOLEY[2,4], A. YAZBACK[5], J. HERNANDEZ[4], J. D. ELSWORTH[6]
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Abstract: MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin precursor to MPP+, which causes permanent symptoms of Parkinson's disease by destroying dopaminergic neurons in the substantia nigra of the brain. Monkeys exposed to low doses of MPTP may show cognitive deficits without motor abnormalities. Such cognitive deficits are similar to that observed in early Parkinson's Disease. The prefrontal cortex, which plays an important role in executive functions, receives a dense dopaminergic input. Dysregulation of dopamine (DA) circuitry may disrupt normal cognition. Using monkeys, the goal of this study was to assess the effects of MPTP neurotoxicity on dendritic circuitry in the monkey dorsolateral prefrontal cortex (DLPFC), a region critical to working memory performance. Young adult African Green monkeys were administered MPTP (2.25mg/kg) over 5 consecutive days. The animals were sacrificed 10 months after drug administration. Blocks of fixed brain tissue were Golgi impregnated and coded slides prepared. Randomly selected layer V and layer II-III pyramids were evaluated for amount of dendritic material and complexity of the dendritic arbors. Analysis of layer II-III pyramids showed a significant loss of arbor in the MPTP-treated monkeys (-22%) with a similar reduction in dendritic branching complexity. Layer V pyramids, by contrast, showed less disruption of circuitry with a 15% loss of dendritic branching and an 11% loss of neuronal complexity. These data support and extend previous findings on spine synapse changes following MPTP. The data is further consistent with a role for dopamine in regulating dendritic branching and spine changes on pyramidal neurons in the dorsolateral prefrontal cortex.

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Poster

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Program#/Poster#: 409.16/J8

Topic: C.03. Parkinson's Disease

Support: Kulhavi Professorship

Title: The utilization of Engrailed 1 in developing neurons for cell replacement therapy in Parkinson's Disease

Authors: S. PARKER[1,2,4], R. WELCHKO[1,2,4], J. ROSSIGNOL[1,2,3,4], M. LU[1,4], *G. L. DUNBAR[1,2,4]
[1]Dept Psychol, Central Michigan Univ., MT PLEASANT, MI; [2]Program in Neurosci., Central Michigan Univ., Mt pleasant, MI; [3]Col. of Med., Central Michigan Univ., Mt. pleasant, MI; [4]Field Neurosciences Inst. Lab. for Restorative Neurol., Mt. pleasant, MI

Abstract: Parkinson's disease (PD) is a neurodegenerative disease with symptoms that include slowness in movement, tremors, depression and in some cases dementia. Cell replacement therapy (CRT) has shown potential to reverse the effects of PD. In PD patients there is a substantial loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc). The aim of CRT is to transplant cells into the human brain that are able to survive for decades, behave similar to SNpc cells, and integrate within the striata. For CRT, finding a method that consistently produces cells with a greater survivability that will effectively replace the lost DA neurons would be invaluable. In this study, a gene involved in the survivability and development of DA neurons, Engrailed 1 (En1), was used in conjunction with three other genes to improve the transformation of mesenchymal stem cells (MSCs) into DA neurons. Previous studies have shown MSCs can be used as a source of DA neurons after exposure to certain genes. This study utilized an adenovirus and retrovirus recently developed in our lab. The adenovirus forces the polycistronic expression of the transcription factors Nurr1, Ascl1, and Lmx1a and the retrovirus forces expression of En1. This study focused on En1 as a candidate for improving the survivability of DA neurons transformed from MSCs. *In vivo* studies have shown En1 is involved in the development of the SNpc, in the generation of DA neurons in the brain, in addition to DA neuron survivability. To confirm that DA neurons were produced, expression levels of specific proteins were tested through RT-PCR and immunocytochemistry (ICC). Survivability of the DA neurons was quantified, plus the efficiency of DA production was quantitatively determined through a technique called high performance liquid chromatography (HPLC). Our results suggests that En1 has the potential for improving the survivability of DA neurons and that techniques to enhance this gene is worthy of further study.

Disclosures: S. Parker: None. **R. Welchko:** None. **J. Rossignol:** None. **G.L. Dunbar:** None. **M. Lu:** None.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.17/J9

Topic: C.03. Parkinson's Disease

Support: Harvard Stem Cell Institute

Consolidated Anti-Aging Foundation

Title: Glucocerebrosidase deficiency and glycolipid accumulation occurs in both normal aging and sporadic Parkinson's disease

Authors: *E. N. MANGANO[1], G. A. SMITH[1], E. PARK[2], H. CAO[2], E. BROWN[2], J. A. BEAGAN[1], Z. E. SCHNEIDER-LYNCH[1], D. A. AHMADI[1], P. J. HALLETT[1], O. ISACSON[1]
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Abstract: Clinical and neuropathological evidence links GBA1, which encodes for the lysosomal hydrolase glucocerebrosidase (GCase) with sporadic PD. GCase is responsible for the conversion of the undegraded lipid substrates glucosylceramide (GluCer) and glucosylsphingosine (GluSph) into ceramide and sphingosine, respectively. A subset of sporadic PD-patients (~4-7%) has been identified as GBA1-mutation carriers, causing diminished activity (~30-40%) of GCase. PD-patients that carry a GBA1 mutation are often diagnosed younger and the symptoms are usually reported as more severe than PD-patients lacking a mutation. Identifying reliable biomarkers that closely associate with the pathogenic changes that occur during the early stages of PD may help increase the accuracy of early diagnosis. Diminished levels of GCase activity have been reported in the brains and CSF of sporadic PD patients, irrespective of whether they harbor GBA1 mutations. Therefore, we hypothesize that GCase activity and its associated glycolipids may be useful biomarkers to predict early stages of sporadic PD. We found widespread GluSph up-regulation in the putamen, cerebellum, hippocampus and frontal cortex, which coincided with reductions in GCase activity of non-GBA mutation carrying sporadic PD-patients in comparison to age-matched control patients. In addition to these changes, we also found age-dependent increases in GluSph, which correspond to a reduction in GCase activity. Moreover, we found age-dependent increases in GluCer and GluSph in wildtype and transgenic mice that overexpress human wildtype α-synuclein. Therefore, we hypothesize that age-dependent dysregulation in GCase activity and accumulation of glycolipids occurs with normal aging, which is further exacerbated with sporadic PD-patients.

Disclosures: E.N. Mangano: None. **G.A. Smith:** None. **E. Park:** None. **H. Cao:** None. **E. Brown:** None. **J.A. Beagan:** None. **Z.E. Schneider-Lynch:** None. **D.A. Ahmadi:** None. **P.J. Hallett:** None. **O. Isacson:** None.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.18/J10

Topic: G.06. Computation, Modeling, and Simulation

Support: R01NS073717-01

Parkinson's Disease Foundation

Title: Quantification of postural stability in patients with Parkinson's disease using mobile technology

Authors: ***S. J. OZINGA**, A. G. MACHADO, A. B. ROSENFELDT, J. L. ALBERTS
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Abstract: Patients with Parkinson's disease (PD) have declines in postural stability and exhibit gait dysfunction. Traditional biomechanical equipment has been used to identify these declines in stability. Unfortunately, the vast majority of clinical environments do not have access to biomechanical equipment to adequately assess postural stability. However, recent advances in processor speed and on-board electronics of mobile devices, present an opportunity to quantify postural stability. The aim of this project was to determine if kinematic data measured by the accelerometer and gyroscope within the iPad was of sufficient quantity and quality to accurately quantify postural stability in patients with PD. Seventeen patients with PD (8 males and 9 females; mean age 62±9) and seventeen healthy age-matched controls (7 males and 10 females; mean age 62±10) completed six different balance conditions under altered surface, stance, and vision. Simultaneous kinematic measurements were gathered from a three-dimensional motion analysis system and the iPad during balance testing. There was agreement between iPad and motion analysis systems in the overall assessment of postural stability for both the PD and older adult population. For each balance trial within the PD population, significant correlation between the two systems was present for: 1) three measures of postural sway from medial-lateral (ML) and anterior-posterior (AP) acceleration time-domain signals, including *peak-to-peak* (r values ranged from 0.75 to 0.97), *normalized path length* (0.49 to 0.99), and *root mean square* (0.71 to 0.98), 2) *95% ellipsoid volume*, combining ML, AP, and trunk rotation (TR) acceleration (0.98 to 1.00), and 3) *total power*, quantifying the magnitude of the ML, AP, and TR resultant acceleration in the frequency-domain (0.94 to 1.00). The tandem stance on firm and foam surfaces showed the greatest discrimination between PD and older adults, with 75% and 100% of significantly different sway measures for the firm and foam surfaces, respectively. Also, PD patients exhibited significantly more postural sway when standing in the tandem stance on a foam surface versus in the double-leg stance on a foam surface ($p<0.05$) as well as standing in the tandem stance on a foam surface versus a firm surface ($p<0.05$). Collectively, these results

indicate the accelerometer and gyroscope hardware within the iPad provide data of sufficient accuracy and quality to discriminate between populations and conditions during postural stability assessments. The objectivity, portability, and ease of use of this device make it ideal for use in clinical environments lacking sophisticated biomechanical systems.

Disclosures: S.J. Ozinga: None. **A.G. Machado:** None. **A.B. Rosenfeldt:** None. **J.L. Alberts:** None.

Poster

409. Parkinson's Disease Models II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 409.19/J11

Topic: G.06. Computation, Modeling, and Simulation

Support: The Academic Medical Organization of Southwestern Ontario

Title: Quantifying the short-term effects of Subthalamic deep brain stimulation surgery on bradykinesia in Parkinson's disease patients

Authors: *M. DELROBAEI[1], G. GILMORE[2], K. OGNJANOVIC[2], F. RAHIMI[1], S. XIAN[1], S. TRAN[2], K. MCISAAC[2], M. JOG[1,2]
[1]Lawson Hlth. Res. Inst., London, ON, Canada; [2]Western Univ., London, ON, Canada

Abstract: Objective: Clinical scale based follow-up of patients undergoing Subthalamic deep brain stimulation (STN-DBS) has shown inconsistent effects on bradykinesia in Parkinson disease (PD) patients. However, quantitative assessment of STN-DBS effect on bradykinesia has not been done. Our group uses multisensory kinematic technologies to study long-term DBS effects on Parkinson disease. We present the kinematic analysis of the short term (3 month) effects of STN-DBS on bradykinesia in 5 PD patients. Method: 5 PD patients (age: 65±3.3, PD duration: 12.2±3.3) were recruited. All patients were eligible for STN-DBS procedure. Each patient was assessed at six time points with both the Unified Parkinson Disease Rating Scale (UPDRS) and a motion capture system: (0) preoperatively, (1) 1 week postoperatively, (2) 2 weeks postoperatively, (3) 1 month following surgery, on-DBS, (4) 2 months following surgery, on-DBS, and (5) 3 months following surgery, on-DBS. The participants performed a standard repetitive forearm pronation-supination task for 5 seconds for each arm while sitting. The angular displacement of forearm was quantified using an Animazoo IGS-180 suit. A set of quantitative measures of bradykinesia were extracted for both arms. The features include: the

root mean square (RMS), the standard deviation (STD), the range of motion (ROM), the dominant frequency (DFQ), and the peak power (PWR). Results: While the bradykinesia score (UPDRS Part III - Item 31) did not show any noticeable changes (0.2, 0.2, 0.2, 0.0, 0.0, and 0.2 on average from baseline to visit five after the surgery), the objective measures identified some improvements. On average, compared to baseline (pre-op), the RMS of forearm angular displacement increased by 23.1, 41.4, 36.6, 14.0, and 36.0% from visit one to visit five after the surgery. The STD increased by 23.9, 32.2, 45.9, 25.7, and 60.9% and the ROM increased by 21.7, 24.6, 23.3, 11.3, and 31.2% for the same duration. The results did not show any consistent increase for the DFQ which slightly increased in the first three visits (11.3, 4.1, 7.0%, respectively), but decreased afterwards (-6.1% at visit 4 and -12.9% at visit 5). Compared to other features, the PWR showed considerably more improvement, as increased by 52.6, 134.5, 155.3, 110.1, and 222.3%, respectively. Conclusions: Using a sensitive tool, our team was able to objectively monitor the immediate effects of STN-DBS on bradykinesia. The microlesion effects were quantified and showed improvements in the proposed features. Although the angular velocity (corresponding to the DFQ), which is clinically more observable, did not generally improve, the peak power showed maximal improvement.

Disclosures: M. Delrobaei: None. **G. Gilmore:** None. **K. Ognjanovic:** None. **F. Rahimi:** None. **S. Xian:** None. **S. Tran:** None. **K. McIsaac:** None. **M. Jog:** None.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.01/J12

Topic: C.03. Parkinson’s Disease

Support: Canadian Excellence Research Chair Program

MJFF

Title: Persistent longitudinal alterations of neurotransmission in LRRK2 G2019S Knock-In mice

Authors: *D. A. BECCANO-KELLY[1], M. VOLTA[1], E. MITCHELL[1], D. SMITH[1], I. TATARNIKOV[1], S. BERGERON[1], K. CO[1], L. MUNSIE[1], H. L. MELROSE[3], M. J. FARRER[1], A. J. MILNERWOOD[2]
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Abstract: Parkinson's disease (PD) is the most prevalent neurodegenerative movement disorder in the world, affecting ~2% of the population. Mutations in the LRRK2 protein are a major cause of familial and sporadic PD. The protein is central to cytoskeletal dynamics and indirectly involved in autophagy, endosomal trafficking (linked to retromer recycling) and exo/endocytosis (synaptic transmission). Changes in neurotransmission appear to be important in PD with most recent studies illustrating that LRRK2 knock-down/knock out influences striatal glutamate signaling. LRRK2 overexpressor (OE) mice also illustrate alterations to short term striatal glutamatergic plasticity in addition to motor deficits and biochemical changes in the levels of key synaptic proteins such as dopamine D2 receptors. In order to investigate the effects of PD related LRRK2 mutations on neurotransmission, a longitudinal study assessing electrophysiological, behavioural and molecular changes was performed on knock-in mice carrying the G2019S mutation (accounting for ~2% of PD cases) in the LRRK2 murine homologue with endogenous control of expression. Whole-cell electrophysiological recordings in acute brain slices from 1, 3, 12 & 18 month old mice revealed varying alterations in glutamatergic transmission onto striatal medium spiny neurones of male heterozygous and homozygous knock-in animals in comparison to wild-type littermates. Concomitant motor phenotype is evident in the same genotypes. Interestingly, pharmacological agents significantly modulate the electrophysiological and behavioural effects of the wild type littermates, whilst having no effect on the animals heterozygous and homozygous for the G2019S mutation. Western blot analysis further revealed altered levels of numerous striatal receptors and signal transduction proteins pertinent to neurotransmission including LRRK2 itself. The data suggest another consequence of the G2019S mutation is altered LRRK2 auto-regulation. The data illustrates an ongoing effect on neurotransmission and behaviour caused by a single PD associated point mutation. These synaptic, motor and cognitive phenotypes are distinct from those observed in either OE or knock-out mice. This neither simplistic loss nor gain of function persists over the mammalian lifetime and represents an on-going effect that may be best tackled therapeutically during the early stage of aberrant neurotransmission alterations, therefore preventing the onset and progression of PD.

Disclosures: D.A. Beccano-Kelly: None. **M. Volta:** None. **E. Mitchell:** None. **D. Smith:** None. **S. Bergeron:** None. **L. Munsie:** None. **H.L. Melrose:** None. **M.J. Farrer:** None. **A.J. Milnerwood:** None. **I. Tatarnikov:** None. **K. Co:** None.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.02/K1

Topic: C.03. Parkinson's Disease

Support: Bluma Tischler Fellowship

Canada Excellence Research Chair Program

Title: Exogenous oligomeric alpha-synuclein in LRRK2 transgenic animals

Authors: *M. VOLTA, S. BERGERON, E. MITCHELL, L. MUNSIE, D. BECCANO-KELLY, A. MILNERWOOD, M. FARRER
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Abstract: Exogenous pathological forms of alpha-synuclein (aSyn) have been reported to seed the formation of intracellular aggregates in neuronal cultures, transgenic mice overexpressing A53T-aSyn and normal mice, through recruitment of endogenous soluble aSyn. *In vivo*, both recombinant aSyn fibrils and brain homogenates from symptomatic A53T-aSyn transgenic mice (exhibiting widespread synucleinopathy) induce progressive neuropathology, neurodegeneration and behavioral alterations. Along with genetic alterations in SNCA, mutations in the LRRK2 gene are amongst the main causes of familial Parkinson's disease (PD). The proteins are reported to interact at the molecular level and in Lewy body disease. However double transgenic mice led to conflicting results. Specifically some data shows LRRK2 overexpression accelerated mutant aSyn-induced pathology whereas other reports showed no additive effects. Thus, the relevance of LRRK2-aSyn interaction *in vivo* remains poorly understood. We sought to employ a more subtle approach, avoiding generalized protein overexpression. Hence, we unilaterally injected LRRK2 KO mice and non-transgenic littermates (NT LMs) into the dorsolateral striatum with either aSyn pre-formed fibrils (PFFs) or PBS vehicle. Animals were assessed before, 30 and 90 days after surgery in various behavioral tests analyzing locomotion, symmetry, anxiety and cognition. We observed a dramatic deficit in recognition memory in NT mice treated with PFFs and we are confirming the effect on KO animals. At the two post-injection time-points, the mice are transcardially perfused with PBS, the brains removed and embedded in paraffin. Immunohistochemistry was performed to assess the phosphorylation of aSyn at the Ser129 residue, the presence of aggregated forms of aSyn (through the use of the pathologic conformation-specific antibody Syn506) and tyrosine-hydroxylase expression in striatum and substantia nigra. To correlate the cognitive impairment with a neurophysiological mechanism, we are also assessing the efficacy of hippocampal plasticity in these animals. In an effort to further characterize the effects of exogenous aSyn, we are treating cortical neuron cultures prepared from LRRK2 KO mice and NT LMs with PFFs or PBS. We will assess pSer129-aSyn and aggregation, together with density of dendritic spines and synaptic markers. Our studies will expand current knowledge on the consequences of amyloid-like protein treatment and clarify the relative roles of LRRK2 and aSyn in neuronal physiology, neuropathology and behavior. These

data will form the basis of a more comprehensive approach on the molecular origins of late-onset PD.

Disclosures: **M. Volta:** None. **S. Bergeron:** None. **E. Mitchell:** None. **L. Munsie:** None. **D. Beccano-Kelly:** None. **A. Milnerwood:** None. **M. Farrer:** None.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.03/K2

Topic: C.03. Parkinson's Disease

Support: 101-2314-B-002-071-MY2

Title: Drug screening for LRRK2 Parkinsonism: using the *Drosophila* dendritic arborization neurons as a model system

Authors: ***C.-H. LIN**[1], H.-I. LIN[2], R.-M. WU[2]
[1]Dept. of Neurology, Natl. Taiwan Universit, Taipei, Taiwan; [2]Dept. of Neurology, Natl. Taiwan Univ. Hosp., Taipei, Taiwan

Abstract: Background and Purpose: Parkinson's disease (PD) is a common neurodegenerative disorder, which is caused by the interplay between genetic and environmental factors. Mutations in Leucine-rich repeat kinase (LRRK2) are the most prevalent in both familial and sporadic PD, especially the non-synonymous G2019S mutation. We have previously created a transgenic LRRK2-G2019S *Drosophila* model and demonstrated loss of dopaminergic neurons, decreased longevity and behavioral deficits, which recapitulates several key features of human parkinsonism. Currently, there is no therapeutic treatment to slow or ameliorate the degeneration process in PD. We proposed to use the LRRK2-G2019S fly model to screen for a library with 640 FDA-approved drugs and identify potential compounds that could have a rescue effect of neurons in this fly model. Methods: An FDA-approved Drug Library consisting of 640 compounds were tested by using dendrite phenotype analysis of 3rd instar larva of LRRK2-G2019S transgenic flies in the first step of our analysis. We tested different concentrations of each compound and each experiment will be replicated for at least 3 times to get the mean value of number of dendritic arbors of each condition. After identifying the potential candidate drugs in the above first step, we will examine the potential beneficial effects on dopaminergic neurons in aging LRRK2-G2019S adult flies. We fed LRRK2-G2019S flies with candidate compounds

two weeks after egg laying and investigated the behavior, life-span and number of dopaminergic neurons of these aging LRRK2-G2019S flies. Results: Among the 640 screened drugs, 29 drugs with potential protective effects were identified using the dendrite phenotype analysis in the first step, with optimal concentration varied between 50 and 100 μM. It was found that 3 out of these 29 drugs demonstrated the most significant rescue effects of dendrite degeneration, those are Flufenamic acid, Lovastatin and Rifampicin. In the second part of the study, we found that treatment with Lovastatin and Rifampicin significantly improved the climbing speed of LRRK2-G2019S aging flies as compared to treatment with DMSO. Treatment with Flufenamic acid only showed some beneficial effect but not reach the significance level. Conclusions: Our results showed that Lovastatin and Rifampicin are potential neuroprotective agents for LRRK Parkinsonism. Future drug trial in patients with LRRK2-G2019S mutations are needed to confirm the beneficial effects of these two medications.

Disclosures: **C. Lin:** None. **H. Lin:** None. **R. Wu:** None.

Poster

410. Parkinson's Disease: Genetic Models

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Program#/Poster#: 410.04/K3

Topic: C.03. Parkinson’s Disease

Support: Telethon Grant GGP12237C

Title: Genetic and pharmacological evidence that G2019S LRRK2 confers hyperkinetic phenotype and prevents motor decline associated with aging

Authors: ***F. LONGO**[1], I. RUSSO[2], D. SHIMSHEK[3], E. GREGGIO[2], M. MORARI[4]
[1]Univ. Degli Studi Di Ferrara, Ferrara, Italy; [2]Dept. of Biology, Univ. of Padova, Padova, Italy; [3]Dept. of Autoimmunity/Transplantation/Inflammation-Neuroinflammation-Novartis Inst. for BioMedical Research, Novartis Pharma AG, Basel, Switzerland; [4]Dept. of Med. Sciences, Section of Pharmacology, and Natl. Inst. of Neuroscience, Univ. of Ferrara, Ferrara, Italy

Abstract: The leucine-rich repeat kinase 2 mutation G2019S in the kinase domain is the most common genetic cause of Parkinson's disease. To investigate the impact of the G2019S mutation on motor activity *in vivo*, a longitudinal phenotyping approach was developed in knock-in (KI) mice bearing this kinase-enhancing mutation. Two cohorts of G2019S KI mice and wild-type littermates (WT) were subjected to behavioral tests, specific for akinesia, bradykinesia and

overall gait ability, at different ages (3, 6, 10, 15 and 19 months). The motor performance of G2019S KI mice remained stable up to the age of 19 months and did not show the typical age-related decline in immobility time and stepping activity of WT. Several lines of evidence suggest that enhanced LRRK2 kinase activity is the main contributor to the observed hyperkinetic phenotype of G2019S KI mice: i) KI mice carrying a LRRK2 kinase-dead mutation (D1994S KD) showed a similar progressive motor decline as WT; ii) two LRRK2 kinase inhibitors, H-1152 and Nov-LRRK2-11, acutely reversed the hyperkinetic phenotype of G2019S KI mice, while being ineffective in WT or D1994S KD animals. LRRK2 target engagement *in vivo* was further substantiated by LRRK2 de-phosphorylation at Ser935 in the striatum and cortex at efficacious doses of Nov-LRRK2-11, and in the striatum at efficacious doses of H-1152. In summary, expression of the G2019S mutation in the mouse LRRK2 gene has beneficial effect on motor function, likely via enhancement of LRRK2 kinase activity. This study provides an *in vivo* model to investigate motor effects of LRRK2 inhibitors.

Disclosures: F. Longo: None. **I. Russo:** None. **D. Shimshek:** None. **E. Greggio:** None. **M. Morari:** None.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.05/K4

Topic: C.03. Parkinson’s Disease

Title: The Parkinsonian LRRK2*R1441G mutation modulates LPS-induced immune response in the brain and periphery

Authors: *E. A. KOZINA, Y. JIAO, Y. DOU, R. J. SMEYNE
St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) are the most frequent cause of familial late-onset Parkinson's disease (PD), with clinical and pathological phenotypes similar to that observed in sporadic PD. The highest level of LRRK2 protein expression is seen in cells of the immune system, particularly in B-cells and macrophages, suggesting that LRRK2 may play an important role in immune signaling pathways. This is supported by findings of genetic variations in LRRK2 in other immunological diseases, including Crohn’s disease and leprosy. Post-mortem human studies of PD brains support a critical role for neuroinflammation in its pathogenesis. Therefore, we compared how the immune system in the brain and periphery

responds in WT and transgenic mice carrying LRRK2*R1441G mutation. To stimulate the immune system, 3 month old transgenic LRRK2*R1441G and FVB/N (WT) mice were injected with saline or lipopolysaccharide (LPS, 5 mg/kg, i.p.). Blood, brain, and spleen tissue were collected at many time points ranging from 1 hour to 7 months after LPS exposure. In these brains we examined microglial activation, cytokine concentration, leukocyte infiltration as well as tyrosine hydroxylase (TH) and LRRK2 protein expression in the striatum and substantia nigra. In the periphery we examined serum cytokines and the number of innate and adaptive immune cells. In the LRRK2*R1441G mice we observed an up-regulation of LRRK2 protein expression in the substantia nigra and cortex after LPS exposure. We also observed a significant increase in the number of activated Iba-1 positive microglial cells in the substantia nigra in both WT and LRRK2*R1441G mice 24 hours after LPS injection. LPS administration also transiently reduced TH expression in the substantia nigra and striatum of LRRK2*R1441G mice but not WT mice. Examination of inflammatory cytokines and chemokines in the substantia nigra and striatum showed higher levels of IL-6, IFN-γ, TNF-α, KC, and MCP-1 in LRRK2*R1441G mice compared to WT. Additionally, the pro-inflammatory cytokine IL-1α was found to be elevated in transgenic LRRK2 mice 2 months after LPS exposure. LRRK2*R1441G mice also showed an infiltration of CD3-positive T-cells into the brain parenchyma. In blood serum, we found that LRRK2*R1441G mice have increased levels of IFN-γ, IP-10, KC, MIP-1α and MCP-1 after the LPS insult. This data suggests that LRRK2*R1441G mice have an enhanced inflammatory response to LPS, and this heightened immune response may contribute to the pathogenesis of R1441G mutation.

Disclosures: E.A. Kozina: None. **Y. Jiao:** None. **Y. Dou:** None. **R.J. Smeyne:** None.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.06/K5

Topic: C.03. Parkinson’s Disease

Title: Merck LRRK2 inhibitor-2 (MLI-2), a potent, selective and centrally active tool compound suitable for exploring the therapeutic potential and safety of LRRK2 kinase inhibition

Authors: *M. J. FELL[1], C. MIRESCU[1], X. ZHOU[2], Y. LIN[2], Z. YIN[1], B. CHEEWATRAKOOLPONG[2], M. SMITH[3], F. POULET[4], C. MARKGRAF[4], L. HYDE[2], M. ELLIS[5], D. DEMONG[6], M. MILLER[6], E. PARKER[7], M. KENNEDY[1], J. MORROW[8]

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Abstract: Mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene cause late-onset autosomal dominant Parkinson's disease (PD) and represents the most common known cause of familial PD. The potential mechanism(s) through which LRRK2 mutations precipitate selective neuronal dysfunction and neurodegeneration in PD is poorly understood. The observation that the most prevalent missense mutation, G2019S, leads to an increase in kinase activity has led to a concerted effort to identify LRRK2 kinase inhibitors as a potential disease modifying therapy for PD. Unfortunately, the ability to interrogate LRRK2 biology with many of the published LRRK2 kinase inhibitors has been limited due to their lack of selectivity over other kinases, poor brain penetration and/or failure to demonstrate CNS in-vivo activity. Here, we characterize the pharmacological properties of MLI-2 a structurally novel, highly potent and selective LRRK2 kinase inhibitor with CNS activity. MLI-2 exhibits single-digit nM potency in an *in vitro* purified LRRK2 kinase assay and a cellular assay monitoring dephosphorylation of LRRK2 pS935 or radioligand competition binding to LRRK2. MLI-2 has excellent kinase selectivity across a range of 308 kinases in addition to a diverse panel of receptors and ion channels. In rodents MLI-2 exhibits good oral bioavailability and is not a Pgp substrate. Acute oral and sub-chronic in-diet dosing with MLI-2 in mice resulted in dose-dependent central and peripheral target inhibition across the 24-hour period as measured by dephosphorylation of LRRK2 pS935. After 15 weeks treatment in mice, MLI-2 produced sustained central and peripheral target engagement (>90% de-phosphorylation of pS935) and was well-tolerated with no evidence of lung or kidney pathology that has been observed in LRRK2 knockout or kinase-dead animals. Together these data demonstrate the suitability of MLI-2 as a tool compound to explore the biology and tolerability of LRRK2 kinase inhibition and therapeutic potential of LRRK2 kinase inhibition in animal models of PD.

Disclosures: **M.J. Fell:** A. Employment/Salary (full or part-time):; Merck. **C. Mirescu:** A. Employment/Salary (full or part-time):; Merck. **X. Zhou:** A. Employment/Salary (full or part-time):; Merck. **Y. Lin:** A. Employment/Salary (full or part-time):; Merck. **Z. Yin:** A. Employment/Salary (full or part-time):; Merck. **B. Cheewatrakoolpong:** A. Employment/Salary (full or part-time):; Merck. **M. Smith:** A. Employment/Salary (full or part-time):; Merck. **F. Poulet:** A. Employment/Salary (full or part-time):; Merck. **C. Markgraf:** A. Employment/Salary (full or part-time):; Merck. **L. Hyde:** A. Employment/Salary (full or part-time):; Merck. **M. Ellis:** A. Employment/Salary (full or part-time):; Merck. **D. DeMong:** A. Employment/Salary (full or part-time):; Merck. **M. Miller:** A. Employment/Salary (full or part-time):; Merck. **E. Parker:** A. Employment/Salary (full or part-time):; Merck. **M. Kennedy:** A.

Employment/Salary (full or part-time):; Merck. **J. Morrow:** A. Employment/Salary (full or part-time):; Merck.

Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.07/K6

Topic: C.03. Parkinson's Disease

Title: Characterization of mitoNEET knock-out mice as Parkinson's disease model

Authors: *W. J. GELDENHUYS[1], L. LIN[1], D. SPEICHER[1], J. YUN[2], R. T. CARROLL[1]
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Abstract: Parkinson's disease is an age-associated neurodegenerative disease which affects the dopaminergic neurons in the *substantia nigra*. The loss of the neurotransmitter dopamine leads to the characteristic movement symptoms seen in Parkinson's disease. Although the etiology is not fully known, the pathology of the neuronal cell death in Parkinson's disease is related to mitochondria dysfunction. The mitochondrial dysfunction leads to reactive oxygen species generation and oxidative stress. mitoNEET is a novel mitochondrial protein which belongs to a zinc-finger group of proteins that is conserved throughout evolution. mitoNEET is an iron-sulfur containing protein located on the outer mitochondrial membrane of mitochondria which regulates mitochondrial bioenergetics, and overexpression of mitoNEET leads to reduction in oxidative stress. Recently, the anti-diabetic drug pioglitazone (a thiazolidinedione) was found to be a ligand for mitoNEET, and has been also shown *in vivo* to be neuroprotective in the MPTP mouse model of Parkinson's disease. We hypothesized that a mitoNEET ligand would be neuroprotective against MPTP-Complex I mediated dopaminergic neuronal cell death. In this study, our goal was to evaluate a mitoNEET ligand NL-1, a derivative of pioglitazone, in the MPTP mouse model. In this study, C57BL/6 aged male mice as well as mitoNEET KO mice were treated with either vehicle, MPTP (35 mg/kg) NL-1 (10 mg/kg) or MPTP and NL-1 for 7 days. After 7 days, the dopamine was evaluated by HPLC-EC and biochemical markers evaluated by Western Blot. These results clearly demonstrated that mitoNEET ligand NL-1 was able to protect the dopaminergic neurons thus suggesting that mitoNEET could be a viable drug target in mitochondrial dysfunction related diseases such as Parkinson's.

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Poster

410. Parkinson's Disease: Genetic Models

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Topic: C.03. Parkinson's Disease

Support: NIH NS063963

Mangurian Foundation

NIH NS073740

Title: Transmission of human Lewy body disease prions to mice

Authors: ***D. R. JONES**[1], D. MARION[1], M. DETURE[1], A. BAINE[1], M. E. MURRAY[1], D. W. DICKSON[1,2], P. J. MCLEAN[1,2]
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Abstract: The synucleinopathies are a group of neurodegenerative disorders characterized by the presence of neuronal inclusions called Lewy bodies and Lewy neurites, and include Parkinson's disease, multiple system atrophy and Lewy body disease (LBD). Recent data suggests that a pathogenic misfolding of the presynaptic protein, alpha-synuclein, and its subsequent aggregation and accumulation is fundamental to the disease process. It is hypothesized that the misfolded isoform is able to induce misfolding of normal endogenous alpha-synuclein, and that the disease propagates from cell-to-cell much like the prion diseases. In this study we performed intracerebral (i.c.) or intraperitoneal (i.p.) inoculations of sarkosyl insoluble or sarkosyl soluble human LBD brain homogenate and show that both non-transgenic mice and mice expressing wild-type human alpha-synuclein develop disease-associated immunopositive deposits at 4 months post-injection (i.c. cohort) or at 8 months post-injection (i.p. cohort). Transgenic mice inoculated i.c. (caudate putamen) displayed a marked increase in proteinase K-resistant LB509 positive deposits in the hippocampus and striatum, and phosphorylated alpha-synuclein positive deposits in the hippocampus, striatum and cortex, compared with transgenic mice inoculated with brain homogenate from an age-matched control having few to no Lewy bodies. Non-transgenic mice inoculated by the i.c. route displayed phosphorylated alpha-synuclein positive

deposits in the hippocampus, striatum and cortex, but no LB509 positive deposits were found. Non-transgenic mice inoculated i.p. had phosphorylated alpha-synuclein deposits in the cerebellum, pons and medulla only. There was no significant difference between the numbers of phosphorylated alpha-synuclein deposits in the rhombencephalon of peripherally inoculated transgenic mice versus control injected transgenic mice. These results provide insight into the mechanisms by which misfolded alpha-synuclein may spread from cell-to-cell, and support the hypothesis that disease-associated alpha-synuclein proteins are true prions.

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Poster

410. Parkinson's Disease: Genetic Models

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Topic: C.03. Parkinson's Disease

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Title: PARK14 Ex2(KO) mice as a novel model for age-dependent Parkinson's disease

Authors: Q. ZHOU[1], A. YEN[1], H. ASAI[2], T. IKEZU[2], B. WOLOZIN[2], *V. M. BOLOTINA[1]
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Abstract: One of the major limitations in Parkinson's disease (PD) study is the absence of a mammalian model that closely mimics age-dependent progression of human PD. Recently, we created a new PARK14 $Ex2^{KO}$ mouse model in which the specific Ca^{2+} signaling function of PARK14 (PLA2g6) was genetically impaired, and discovered that ageing $Ex2^{KO}$ mice develop a strong PD-like phenotype. Here we validate PARK14 $Ex2^{KO}$ mice as a new model for age-dependent PD, and present *in vivo* and *in vitro* evidence for progressive loss of dopaminergic (DA) neurons in substantia nigra pars compacta (SNpc) and PD-like motor dysfunction in $Ex2^{KO}$ animals. Comparative analysis of ageing $Ex2^{KO}$ and WT male littermates (using balance beam, pole, rotarod, open field and other behavioral tests) uncovered the onset of ataxia and motor deficit at 10 months, which slowly progressed with age, and reached very severe motor dysfunction by 20 months. Balance beam test showed that the number of missteps in $Ex2^{KO}$

animals was 3±1, 4±1, 16±3 and 30±4 -fold higher than in WT animals at age of 10, 12, 16 and 20 months, respectively. Pole test showed 5±1 and 7±1 -fold reduction in performance of $Ex2^{KO}$ mice at 12 and 16 months, respectively. Open Field test revealed that $Ex2^{KO}$ mice display increased stereotypic movement, and decreased rearing. Importantly, L-DOPA reversed the motor deficits in KO^{Ex2} mice in dose and age-dependent manner: single injection of 5, 10, and 25 mg/kg L-DOPA improved motor coordination in 16 months old mice by 18%, 60%, and 80%, respectively. Lower doses of L-DOPA achieved similar effects in younger (12 months), while higher doses were needed for older (20 months) animals. It is important to mention that progressive ataxia and age-dependent motor dysfunction in $Ex2^{KO}$ animals were not a result of muscle or neuroaxonal dystrophy, as grip test showed no difference in $Ex2^{KO}$ and WT animals up to 20 months of age. Stereological analysis of TH-positive neurons in SNpc revealed progressive loss of dopaminergic neurons in ageing $Ex2^{KO}$, but not WT animals. However,no difference was observed in the number of TH+ neurons at 6-8 months, about 35% reduction could be seen in $Ex2^{KO}$ at 16 months, and more than 55% reduction at 24 months. Importantly, the number of degenerating PAS+ neurons in the SNpc was increased more than 10-fold, but no difference was found in the hippocampus or motor cortex of 16 months old $Ex2^{KO}$, compared to WT animals. Thus, $Ex2^{KO}$ mice develop age-dependent neurodegeneration with progressive loss of DA neurons in SNpc and specific motor dysfunction that closely mimic parkinsonism in humans. This mouse offers a novel mammalian model of age-dependent PD that can be used for testing new drugs and treatments.

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Poster

410. Parkinson's Disease: Genetic Models

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Michael J. Fox Foundation for Parkinson’s Disease Research

American Parkinson's Disease Association

Title: LRRK2-G2019S expression in the brains of BAC transgenic rats resembles the distribution of LRRK2 in humans and non-human primates

Authors: *J. BLACKBURN[1], R. COWELL[2], J. P. L. DAHER[2], M. S. MOEHLE[2], K. M. HINKLE[3], H. L. MELROSE[3], D. G. STANDAERT[2], A. B. WEST[2], L. A. VOLPICELLI-DALEY[2]
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Abstract: A major percentage of late-onset Parkinson's Disease (PD) patients have mutations in leucine-rich repeat kinase 2 (LRRK2). Here, we utilize recently characterized monoclonal antibodies to evaluate rodent LRRK2 expression in the cortex, striatum, and substantia nigra pars compacta, brain regions relevant to PD. In both mice and rats, LRRK2 is highly expressed in the cortex and striatum, particularly in pyramidal neurons of layer V and in medium spiny neurons within striosomes. Nontrangenic rats express minimal to no LRRK2 in the SNpc. However, LRRK2-G2019S expression derived from human BAC constructs causes LRRK2 to be expressed in the substantia nigra pars compacta. In addition, unlike nontransgenic rats, BAC-LRRK2-G2019S rats express LRRK2 in striatal cholinergic interneurons. The distribution of LRRK2 from human BAC constructs more closely resembles descriptions of LRRK2 in humans and non-human primates. Computational analyses of DNA regulatory elements in LRRK2 show a primate-specific promoter sequence which may direct neuronal expression patterns. Together, these studies will aid in understanding the normal function of LRRK2 in the brain and will assist in model selection for future studies.

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Poster

410. Parkinson's Disease: Genetic Models

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Topic: C.03. Parkinson's Disease

Title: Transgenic mice overexpressing familial Parkinson's disease mutations in protein translation factor eIF4G1 exhibit selective neurodegeneration of dopamine neurons

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Abstract: Eukaryotic translation initiation factor gamma 1 (eIF4G1) is involved in protein translation. Recent studies identified point mutations in the EIF4G1 gene in autosomal dominant Parkinson's disease (PD), potentially linking eIF4G1 and protein translation with PD. Recent finding from our laboratory show that the alterations in protein translation plays a critical role in neurodegeneration in LRRK2 model of PD. To explore the role of eIF4G1 in PD, we generated conditional eIF4G1 transgenic mice expressing wild type and disease associated mutants (R1205H or A502V) eIF4G1. These mice express high levels of eIF4G1 in the olfactory bulb, cortex, hippocampus, substantia nigra pars compacta (SNpc) and striatum. When disease associated mutant eIF4G1 is expressed in brains of transgenic mice, they develop progressive degeneration of dopaminergic neurons in the SNpc, and exhibit behavioral deficits that are not observed in wild type eIF4G1 or littermate control mice. These data implicate these mutations in eIF4G1 play a role in the pathogenesis of PD, potentially through alterations in protein translation. Further characterization of these mice will reveal crucial roles of protein translation and eIF4G1 mutations in the pathogenesis and progression of PD.

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Poster

410. Parkinson's Disease: Genetic Models

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CUNY (graduate center)

Title: Parkin modulation by inflammation and mitochondrial impairment leading to caspase- and calpain-dependent cleavage: cAMP neuroprotection and relevance to Parkinson disease

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Abstract: Parkin is an E3 ubiquitin ligase that provides neuroprotection by regulating proteasomal degradation of cytoplasmic proteins, mitochondrial dysfunction via mitophagy, and the targeting of specific mitochondrial proteins for proteasomal degradation. Parkin dysfunction due to mutations or to post-translational modifications is associated with Parkinson disease (PD). Using rat midbrain and cerebral cortical cultures, we investigated changes in Parkin induced by inflammation and mitochondrial impairment, as both are involved in the etiology of PD. As such, we found that the effects of the neurotoxic product of inflammation prostaglandin J2 (PGJ2) and the mitochondrial inhibitor oligomycin (Oligo) on Parkin integrity were similar with both types of cultures. PGJ2 induced Parkin cleavage by caspase, while Oligo induced calpain cleavage of Parkin. Whereas Parkin cleavage by caspase has been shown to result from different stress conditions, calpain cleavage of Parkin has not been reported. Our results show that calpain-cleaved Parkin migrates as a doublet with a size that differs from the caspase-cleaved fragment induced by PGJ2. The higher molecular weight form of the doublet may be due to phosphorylation. We also determined that full length (FL) Parkin is localized to mitochondria and the cytoplasm regardless of cellular conditions, while calpain or caspase-cleaved forms of Parkin are mostly detected in the mitochondrial fraction. The cleavage of Parkin by caspase and calpain seems to occur at a site that frees its UbL domain, to generate a fragment that could act as a proteasome inhibitor. Since PGJ2 and Oligo are neurotoxic and induce Parkin cleavage, we attempted to preserve Parkin integrity by raising intracellular cAMP with the lipophilic peptide PACAP27 (pituitary adenylate cyclase activating peptide). We chose this approach because the cAMP signaling pathway regulates the proteasome and is also known to mediate neuroprotection. We show that PACAP27 prevents the caspase- but not the calpain-dependent cleavage of Parkin. By using an alternate approach, we found that stabilizing intracellular ATP levels with the creatine analog cyclocreatine prevents Parkin cleavage by calpain. We also established that the phosphatase inhibitor okadaic acid stabilizes a higher molecular weight form

of FL Parkin and reduces its calpain-cleaved form. These findings suggest that stabilizing FL Parkin phosphorylation by phosphatase inhibition decreases its susceptibility to calpain-cleavage. Overall, characterizing mechanisms that stabilize Parkin integrity is important to provide novel therapeutic strategies for treating PD.

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Poster

410. Parkinson's Disease: Genetic Models

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Topic: C.03. Parkinson's Disease

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Title: PKCgamma knockout animal is a potential model for Parkinsonian syndrome: The role of betaPIX phosphorylation at Ser340 and Ser583 in dopamine release

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Abstract: Protein kinase C (PKC) has been implicated in the control of neurotransmitter release. The AS/AGU rat, which has a nonsense mutation in PKCγ shows Parkinsonian-like symptoms, including dopamine (DA) release impairment in the striatum. Here, we found that PKCγ protein was abolished in AS/AGU rat and that PKCγ-knockout(KO) mice showed Parkinsonian syndrome, suggesting that PKCγ-KO animals are possible models for Parkinsonian syndrome. To address this issue, we attempted to identify PKCγ substrates responsible for the regulated exocytosis of DA *in vivo*. For this purpose, we employed PKCγ-KO mice for phosphoproteome analysis by using Hydroxy Acid-Modified Metal Oxide Chromatography method. We found 10 candidates proteins with PKC phosphorylation motif that showed the decreased level of phosphorylation in the striatum of PKCγ-KO mice. Among them, we focused on Pak-interacting

exchange factor-β (βPIX), which is a Cdc42/Rac1 guanine nucleotide exchange factor, and found that PKCγ directly phosphorylates βPIX at Serine 583 and indirectly at Serine 340 in cell culture. We also found that PKC phosphorylated βPIX *in vivo*. Classical PKC inhibitors and βPIX knockdown (KD) significantly suppressed the Ca2+-evoked DA release in PC12 cells. The decreased level of DA release in βPIX KD cells was fully rescued by wild-type (WT) βPIX, but not βPIX mutants whose serine 340 or serine 583 was substituted by alanine (S340A or S583A). Double KD of Cdc42 and Rac1 decreased DA release of PC12 cells. Furthermore, the interaction of Cdc42/Rac1 with phospho-mimicking mutants of βPIX, S340E or S583E, was stronger than that with WT βPIX. These findings indicate that the phosphorylation of βPIX at Serine 340 and Serine 583 has pivotal roles in Ca2+-evoked DA release through interaction with Cdc42/Rac1 in the striatum. Thus, we propose that PKCγ positively modulates DA release through the β2PIX phosphorylation. The PKCγ-βPIX-Cdc42/Rac1 phosphorylation axis may provide a new therapeutic target for the treatment of Parkinsonian syndrome.

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Poster

410. Parkinson's Disease: Genetic Models

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A.S. has a fellowship from the UPV/EHU

Title: Effect of buspirone on the subthalamic nucleus on an animal model of parkinson´s disease: An electrophysiological study

Authors: A. SAGARDUY[1], J. LLORENTE[1], C. MIGUELEZ[2,1], T. MORERA-HERRERAS[1], A. ARISTIETA[1], *J. RUIZ-ORTEGA[2,1], L. UGEDO[1]
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Abstract: INTRODUCTION: L-DOPA is the most effective treatment for Parkinson's disease, but prolonged use induces motor complications including dyskinesia. Although the mechanisms underlying the development of L-DOPA-induced dyskinesia are not completely understood, prior studies demonstrated the implication of the subthalamic nucleus (STN). It has been described that buspirone, elicits an antidyskinetic effect. The aim of this study was to evaluate the effect of buspirone on STN neuron activity. METHODS: Single-unit extracellular recordings were performed *in vivo* on STN neurons from four different groups, i.e., control, chronically treated with L-DOPA, lesioned and lesioned chronically treated with L-DOPA (dyskinetic) rats. Also, preserving the STN-projections as intact as possible, *in vitro* cell-attached recordings were performed in 500 µm-thick, 3º angle parasagittal slices including the STN from naïve rats. RESULTS: In control rats buspirone administration decreased the firing rate in a dose-dependent manner (4 mg/kg i.p. 35% and 8 mg/kg i.p. 67%) and the coefficient of variation resulted increased. Similar results occurred in sham L-DOPA rats (8 mg/kg i.p. 58%). Buspirone (8 mg/kg i.p.) has not elicited any effect on STN neuron activity when was administered in rats with nigrostriatal degeneration. In addition, in control rats WAY-100635, a selective antagonist of 5HT1A receptors, and PD128907, a selective agonist of D3 receptors, blockaded the effect of buspirone and this effect was reversed by WAY-100635 but not by PD128907. Furthermore, the acute and chronic administration of L-DOPA (6 mg/kg plus benserazide 12 mg/kg i.p.) did not modify the inhibitory effect of buspirone (4 mg/kg i.p.). Conversely, in parasagittal slices containing the STN, buspirone induced excitatory, inhibitory and also biphasic responses being only the inhibitory effect prevented by WAY-100635. CONCLUSION: Buspirone administration *in vivo* induces a reduction on the firing rate of the STN neurons through 5HT1A and D3 receptors whereas *in vitro* buspirone seems to show a more variable effect. Moreover, effect of buspirone was abolished in 6-OHDA lesioned rats, suggesting that the STN may not be directly involved in its antidyskinetic effect.

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Poster

410. Parkinson's Disease: Genetic Models

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Topic: C.03. Parkinson's Disease

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Title: Investigating the dual effect of dopamine transporter over-expression and vesicular monoamine transporter 2 under-expression in genetically modified mice

Authors: *S. T. MASOUD[1], A. J. RAMSEY[1], G. W. MILLER[2], A. SALAHPOUR[1]
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Abstract: The dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) are two key proteins that regulate dopamine (DA) homeostasis. DAT takes up extracellular DA into the presynaptic neuron, while VMAT2 sequesters intracellular DA into vesicles. Both proteins play an important role in maintaining cytosolic levels of DA. Accumulation of cytosolic DA has been shown to propagate oxidative stress through metabolism, autoxidation and enzyme dependent reactions. Therefore, we hypothesized that any alteration in the transport and packaging of DA that leads to its cytosolic accumulation will result in neurotoxicity due to increased oxidative stress. Previously, we generated transgenic mice that selectively over-express DAT in DA neurons (DAT-tg mice). These animals display 36% loss of DA neurons, possibly as a result of increased DA reuptake. Additionally, we obtained VMAT2-knockdown (VMAT2-kd) mice that express only 5% of normal VMAT2 levels and show similar neurodegeneration as the DAT-tg mice. In this study, we intercrossed DAT-tg and VMAT2-kd mice to produce double transgenic DAT-tg/VMAT2-kd mice. These mice are hypothesized to display greater buildup of cytosolic DA than either genotype alone, due to the dual effect of higher DA reuptake (DAT over-expression) combined with impaired DA vesicular storage (VMAT2 knockdown). Preliminary characterization of DAT-tg/VMAT2-kd mice reveals low striatal DA tissue content and higher metabolite to DA ratios, which could reflect an increase in DA turnover. Interestingly, DAT-tg/VMAT2-Kd mice are 5 times more active than WT animals in open field locomotion. To evaluate if this hyperactivity is an indication of DA receptor supersensitivity, we treated these mice with apomorphine, a non-selective D1/ D2 receptor agonist. DAT-tg/VMAT2-kd mice showed increased stereotypic behavior in response to apomorphine, suggesting enhanced DA receptor function. Next, we treated the mice with different doses of amphetamine. At 1 mg/kg, DAT-tg/VMAT2-kd mice were hyperactive, however at 2 mg/kg, they displayed peculiar dyskinetic movements (tremor, jerking). These results suggest that DAT-tg/VMAT2-kd mice are highly sensitive to amphetamine. Future experiments with these mice include 1) stereology to determine the extent of neurodegeneration 2) radioligand binding to assess receptor levels and 3) response to other dopaminergic drugs (cocaine, L-DOPA). These studies will shed light on the role of DAT, VMAT2 and cytosolic DA in causing neurotoxicity *in vivo*. Since DA neurons are exceptionally vulnerable to damage in Parkinson's disease, it is possible that the mishandling of DA could act as a risk factor for these cells.

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Poster

410. Parkinson's Disease: Genetic Models

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 410.16/L6

Topic: C.03. Parkinson's Disease

Support: MJF Foundation

NIH grant NS052818

Title: Effects of overexpressed transcription factors in the locus coeruleus on dopamine phenotypes in the brain of Parkinson's mouse models

Authors: ***M.-Y. ZHU**, K. CUI, F. YANG, Y. FAN, D. PETERSON, B. CUMMINS, R. BROWN
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Abstract: Dysfunction of the noradrenergic system is one of the pathogenetic alterations of Parkinson's disease, which may also influence the functional status of the dopamine system. However, their correlation remains to be elucidated. Phox2, Hand2 and Gata3 are the transcription factors that have been found to play a role in the maintenance of noradrenergic function in the mature brain. In the present study, we examine the possible effect of overexpression of these transcription factors in the locus coeruleus on the expression of the dopamine phenotypes in VMAT2 Lo mice, a mouse model of Parkinson's disease. cDNAs of mouse Phox2a/2b, Hand2 and Gata3, which have been constructed in the lentiviral vectors, were microinjected into the locus coeruleus, either alone or in combination, of 6 and 12 month-old model mice. The expression of these transcription factors as well as dopamine β-hydroxylase (DBH) and tyrosine hydroxylase (TH), along with behavioral performance on anxiety and cognition were measured. Results showed that overexpression of these transcription factors in the locus coeruleus significantly affected expression of TH and DBH in the striatum, substantia nigra, hippocampus or frontal cortex, respectively. Microinjection with Phox2a/2b, Gata3 Hand2 alone markedly increased TH protein levels in the striatum, substantial nigra and frontal cortex of 6 month-old model mice, as compared to non-treated VMAT2 Lo mice and age-matched wild-type littermates. Furthermore, injection with these transcription factors, alone or in combination, resulted in a very similar result in 12 month-old model mice. Moreover, injection with Phox2a/2b and Gata3, as well as combination injection of Phox2a/2b and gata3 or Phox2a/2b and Hand2 significantly alleviated cognitive deficits on the Morris water maze. Injection with Gata3 or Phox2b in combination of Gata3 significantly diminished latency to explore the open

arms on the elevated T-maze, a test of anxiety. The present study demonstrates an upregulatory effect of overexpression of transcription factors in the locus coeruleus on the functional status of central dopamine system, which may provide some clues for improving therapeutic strategy for Parkinson's disease. (Supported by MJFF foundation and NIH grant NS052818)

Disclosures: M. Zhu: None. **K. Cui:** None. **F. Yang:** None. **Y. Fan:** None. **D. Peterson:** None. **B. Cummins:** None. **R. Brown:** None.

Poster

410. Parkinson's Disease: Genetic Models

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: NIH/NINDS NS077022

Gardner Family Center for Parkinson's Disease and Movement Disorders

Title: The effect of chronic manganese administration in Atp13a2-deficient mice

Authors: *S. S. KARKARE[1,2], N. SANTIAGO[3], S. PAMPHILE[3], O. R. EKHATOR[3], A. M. LEHMKUHL[1], A. K. CLIPPINGER[5], S. LINN[5], B. LIOU[6], Y. SUN[6], G. E. SHULL[4], P. SCHULTHEIS[5], S. M. FLEMING[3,2]
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Abstract: Mutations in the gene ATP13A2 are associated with Kufor-Rakeb Syndrome and Neuronal Ceroid Lipofuscinosis. The former is designated as a rare inherited form of Parkinson's disease (PD). The function of ATP13A2 is unclear but *in vitro* studies suggest that it is involved in the lysosomal degradation of proteins and in the homeostasis of manganese and zinc. We recently showed that ATP13A2-deficient (13a2) mice develop age-dependent sensorimotor deficits, and enhanced lipofuscinosis and accumulation of insoluble alpha-synuclein (aSyn) in the brain. The presynaptic protein aSyn abnormally accumulates in PD and is degraded through lysosomal-autophagy degradation pathways suggesting the relationship between ATP13A2 and aSyn may be important in PD. In the present study, we hypothesized that loss of ATP13A2

function combined with chronic manganese administration in mice would lead to alterations in sensorimotor function, increased and accelerated lipofuscin pathology and accumulation of insoluble aSyn in the brain. Wildtype (WT) and 13a2 mice received daily intraperitoneal injections of either vehicle or 5 mg/kg of a manganese solution for 45 days. Separate cohorts of WT and 13a2 mice were treated at 5-9 months (young) or at 12-19 months (old) of age. On day 30 all mice were tested on a battery of sensorimotor tests including the challenging beam traversal test, spontaneous activity in the cylinder, and gait. Lipofuscin autofluorescence was measured in multiple brain regions including the cortex, hippocampus, cerebellum, and substantia nigra. Soluble and insoluble aSyn protein levels were measured in the ventral midbrain. We found that older 13a2 mice receiving manganese displayed a significant increase in motor activity on the challenging beam and in spontaneous activity compared to 13a2 receiving vehicle. There were no significant differences in gait. Lipofuscin autofluorescence in the substantia nigra was significantly increased in older 13a2 mice receiving manganese compared to 13a2 mice treated with saline. In addition, insoluble aSyn was significantly increased in the ventral midbrain in older manganese-treated 13a2 mice compared to vehicle-treated 13a2 mice. These results indicate that loss of ATP13A2 function leads to an increased sensitivity to manganese *in vivo*.

Disclosures: **S.S. Karkare:** None. **N. Santiago:** None. **S. Pamphile:** None. **O.R. Ekhator:** None. **A.M. Lehmkuhl:** None. **A.K. Clippinger:** None. **S. Linn:** None. **B. Liou:** None. **Y. Sun:** None. **G.E. Shull:** None. **P. Schultheis:** None. **S.M. Fleming:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.01/L8

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation for Parkinson's Research

Title: Novel mouse models with Parkinson's disease-related autophagy deficits

Authors: ***M. SASNER**[1], T. N. MARTINEZ[2], K. D. DAVE[2], M. J. FARRER[3], W. D. HIRST[4], S. CLARK[5], M. A. FRASIER[2]
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Abstract: Improved animal models of Parkinsonism are essential to advance our understanding of disease pathophysiology and for eventual testing of potential therapeutics. To that end, the Michael J. Fox Foundation (MJFF) has funded the generation and characterization of novel animal models of Parkinsonism as part of its strategy to provide preclinical tools to the Parkinson's disease (PD) research and drug development community with minimal barriers. Various models expressing mutations in *SNCA, LRRK2, Park2, Park7* (DJ-1), and *Pink1* are available. One area of focus has been the creation of models with defects in pathways responsible for protein trafficking and degradation, as mutations in these pathways have been associated with PD incidence in patient populations. Human carriers of certain mutations in the *GBA* gene are at increased risk of developing PD. *GBA* encodes lysosomal glucocerebrosidase (GCase); mutations causing functional deficits in this lysosomal enzyme cause aberrant accumulations of glucosylceramide and glucosylsphingosine and are believed to also generally perturb lysosomal degradation pathways, potentially leading to toxic accumulation of α-synuclein, which is itself implicated both genetically and neuropathologically in PD. The GCase enzyme produced by the D409V mutation is catalytically active, but unstable. The novel homozygous *Gba* D409V knock-in mouse model described herein will be characterized for behavioral and pathological phenotype as well as for levels of α-synuclein and GCase activity at 4, 8, and 12 months of age. As part of the effort to the effort to understand the role of the *GBA* D409V point mutation, the mouse knock-in model crossed to a SNCA over-expressing transgenic line will be assayed for PD phenotypes. Other autophagy-deficient models relevant to PD include an *Atp13a2* knockout, which exhibits alpha-synuclein accumulation and sensorimotor deficits, and a new *Vps35* D620N knock-in which can be used to create a conditional knockout. These models, along with many others, are available with no licensing fees for basic research and therapy development efforts from The Jackson Laboratory PD mouse model resource. Availability and phenotypic characterization data will be presented at http://research.jax.org/grs/parkinsons.html .

Disclosures: M. Sasner: None. **T.N. Martinez:** None. **K.D. Dave:** None. **M.J. Farrer:** None. **W.D. Hirst:** A. Employment/Salary (full or part-time):; Pfizer. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer. **S. Clark:** A. Employment/Salary (full or part-time):; Amicus Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amicus Therapeutics. **M.A. Frasier:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.02/L9

Topic: C.03. Parkinson's Disease

Title: Characterization, comparison, and cross-validation of *in vivo* alpha-synuclein models of parkinsonism

Authors: *T. N. MARTINEZ[1], M. SASNER[2], M. T. HERBERTH[3], R. C. SWITZER, III[4], S. O. AHMAD[5], K. C. LUK[6], S. RAMBOZ[7], A. E. KUDWA[7], D. KIRIK[8], J. FLORES[8], R. J. MANDEL[9], M. P. GETZ[9], R. BROWN[9], R. J. SAMULSKI[10], J. C. GRIEGER[10], D. DISMUKE[10], S. S. DAS[11], M. A. FRASIER[1], K. D. DAVE[1]
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Abstract: Alpha-synuclein is a 140 amino acid protein implicated both genetically and neuropathologically in Parkinson's disease (PD). Mutations in the *SNCA* gene that encodes the alpha-synuclein protein are associated with heritable PD. Increased levels of alpha-synuclein lead to neurodegeneration both *in vitro* and *in vivo* and aggregated alpha-synuclein is the primary component of Lewy bodies, the histopathological hallmark of PD. Thus, therapeutic strategies aimed at reducing alpha-synuclein and the development of *in vivo* models of alpha-synucleinopathy are areas of active interest in the PD field. The Michael J. Fox Foundation (MJFF) accelerates progress in PD research and therapeutic development by generating and rigorously characterizing preclinical tools and animal models and subsequently providing them to the research community with minimal barriers. Here we describe MJFF efforts to compare *in vivo* models of alpha-synucleinopathy. Data from a MJFF-sponsored side-by-side evaluation of five different transgenic alpha-synuclein mouse models for molecular, behavioral, neurochemical, and pathological phenotypes at three different ages (4, 8, and 12 months of age) will be presented. In addition, the MJFF supported a cross-validation study of the alpha-synuclein transmission mouse model of neuropathology (Luk K.C. et al., *Science* 2012). In this

validation study, behavioral and pathological phenotypes were examined at 30, 90, and 180 days post stereotaxic injection of alpha-synuclein fibrils into the dorsal neostriatum of mice. Moreover, the MJFF generated and characterized adeno-associated virus (AAV) vectors (serotypes 2 and 5) expressing human wild-type (WT) alpha-synuclein or GFP control. Here we report the *in vivo* characterization results for these alpha-synuclein AAV2 and AAV5 vectors in Sprague-Dawley rats, demonstrating efficient transduction within the rat substantia nigra and increased levels of human WT alpha-synuclein *in vivo*. Taken together, these data can help inform the PD community of the utility and reproducibility of various *in vivo* rodent models of alpha-synucleinopathy and may be of value to researchers seeking appropriate models in which to test potential therapeutics targeting alpha-synuclein. We are eager to share these data with the PD community and to facilitate additional characterization and distribution of these important preclinical models of parkinsonism.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01NS082205

Adrienne Helis Grant

Dianna Helis Grant

Title: Characterization of a novel mouse model designed to study the mechanistic link between PD-associated GBA mutations and α-synucleinopathies

Authors: *D. KIM[1,2,6], S. KWON[1,7], S. CHOI[1,2,6], V. L. DAWSON[1,2,3,4,8], T. M. DAWSON[1,2,5,4,8], H. S. KO[1,2,6]
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Abstract: Heterozygous mutations in GBA represent one of the most frequent genetic risk factors for Parkinson's disease (PD) and Dementia with Lewy bodies (DLBs). Converging evidence suggests that reduced GBA enzyme stability, defective intrinsic enzyme activity, and compromised glucosylceramide metabolism may contribute to the degenerative process of sporadic PD. Neuropathological and clinical studies of PD patients with GBA mutations revealed that the mutations are strongly associated with α-synuclein accumulation, ultimately resulting in the degeneration of dopaminergic (DA) neurons and the presence of LB pathology. This suggests that biochemical defects caused by GBA mutations may play a pivotal role in pathogenesis of PD and DLBs. However, the specific mechanism underlying the interaction between α-synuclein and the pathogenic mutant forms of GBA remains elusive. To examine the pathological interaction between α-synuclein and GBA *in vivo*, we have generated bigenic mice by crossing D409H mutant GBA knock-in mice with the human A53T mutant α-synuclein transgenic (hA53T α-syn Tg) mice. Different genotype cohorts were collected, aged, and then assessed for their life-span, behavior, neuronanatomical and neurochemical changes. Based on the survival analysis, the life-span of the hA53T α-syn Tg with 29% enzyme deficiency due to the expression of a heterozygous D409H mutation in GBA was significantly shortened compared to that of the hA53T α-syn Tg. More dramatic reduction of the life-span was illustrated in hA53T α-syn Tg with the 61% enzyme deficiency caused by the expression of homozygous D409H GBA mutant. In addition, approximately 20% loss of DA neurons was shown in the D409H GBA-Het/hA53T α-syn Tg mice, which was exacerbated by the expression of homozygous D409H GBA in the hA53T α-syn Tg mice at 6 months of age. Ongoing experiments are designed to further assess the accumulation of detergent-insoluble A53T α-synuclein species and the behavior abnormalities. In part, our results suggest that deficiency of GBA due to D409H mutation plays a significant role in the pathogenesis of A53T α-synuclein-induced neurodegeneration, thus maintaining GBA in a catalytically active form may be an efficient therapeutic approach for the treatment of PD and α-synucleinopathies.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation SYN Tap 2013

EVER Neuro Pharma

Title: Biophysical characterization of the interaction of NPT200-11 with alpha-synuclein

Authors: *B. SZOKE[1], W. WRASIDLO[2], E. STOCKING[1], I. TSIGELNY[2], T. C. SCHWARTZ[3], R. KONRAT[3], A. D. PAULINO[1], D. L. PRICE[1], S. WINTER[4], E. MASLIAH[2], D. BONHAUS[1], D. MEIER[1,4]
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Abstract: Neuropore Therapies, Inc. is developing novel therapeutics for the treatment of Parkinson's disease and related neurodegenerative disorders by targeting the specific aberrant forms of α-synuclein (ASYN) that play a key role in the pathogenesis of these disorders. Candidate compounds such as NPT200-11, which are designed to stabilize conformations of ASYN incapable of propagating into toxic oligomers have been evaluated in animal models and shown to have beneficial effects, reducing the accumulation of ASYN, normalizing markers of neurodegeneration and improving motor function. In the current studies we utilized molecular modeling and simulation along with a variety of biophysical assessment tools including NMR spectroscopy, fluorescence polarization based aggregation assays and electron microscopic visualization of ASYN oligomers to more fully characterize the interaction of NPT200-11 with ASYN and the consequent effects on the formation of ASYN oligomers in lipid membranes. Data from these studies clearly demonstrate that NPT200-11 alters the aggregation of ASYN and prevents the formation of specific oligomeric structures believed to contribute to the neurotoxicity of misfolded oligomerized ASYN in Parkinson's disease.

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intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. F. Consulting Fees (e.g., advisory boards); Neuropore Therapies, Inc. **R. Konrat:** 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.; Neuropore Therapies, Inc. **A.D. Paulino:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **D.L. Price:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **S. Winter:** A. Employment/Salary (full or part-time):; EVER Neuro Pharma. **E. Masliah:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. F. Consulting Fees (e.g., advisory boards); Neuropore Therapies, Inc. **D. Bonhaus:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **D. Meier:** A. Employment/Salary (full or part-time):; EVER Neuro Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc, EVER Neuro Pharma. **T.C. Schwartz:** 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.; Neuropore Therapies, Inc.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.05/L12

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

NIH-AG18840

Neuropore Therapies

Title: Live imaging of alpha-synuclein aggregates in the retina of alpha-synuclein-GFP transgenic mice as a marker of the alpha-synuclein pathology in the brain

Authors: *E. M. ROCKENSTEIN[1], D. L. PRICE[4], D. BONHAUS[4], M. MANTE[1], J. D. LINDSEY[2], E. MASLIAH[3]
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Abstract: Abnormal accumulation of alpha-synuclein has been reported to play an important role in neurodegeneration and synaptic dysfunctional in Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). Real time visualization and monitoring of alpha-synuclein accumulation in the central nervous system (CNS) is critical in order to better understand the mechanisms of neurodegeneration. We have previously described a transgenic model expressing the alpha-synuclein-GFP fusion protein under the PDGF-beta promoter, and have shown that this model allows *in vivo* monitoring of the dynamics of alpha-synuclein accumulation in the brain utilizing two-photon microscopy. We have now expanded these studies to investigate whether the alpha-synuclein-GFP in these mice also accumulates in the retina and whether this accumulation mirrors that observed in the brain. Retinal imaging can provide a non-invasive means to evaluate optical pathology (retinal fundal maps and optical coherence tomography of vasculature and fluorescent-tagged proteins). For this purpose alpha-synuclein-GFP transgenic mice were imaged at various intervals with the Phoenix Micron III Retinal Imaging Microscope (Phoenix Research Labs, Pleasanton, CA). Mouse bright field image retinal maps (normal scan mode) were acquired followed by fluorescent images (progressive scans of 30) in the same orientation for each eye. Similar to the observations in the brain, in the retina of the tg mice we observed progressive accumulation of alpha-synuclein-GFP deposits in the perivascular region and in punctae around blood vessels likely associated with nerve terminals, retinal ganglion cells (RGC's) and glia. Double immunocytochemical studies are underway to more precisely identify the cells in the retina accumulating alpha-synuclein-GFP. In addition , imaging studies of RGC's by confocal scanning laser microscopy mode with a Spectralis imager (Heidelberg Engineering, Carlsbad, CA) with modified optics enabling us to focus on the mouse retina and by AlexaFluor594-conjugated cholera toxin B subunit (AFCTB) injected into the superior colliculus. These observations correlate with those reported in the CNS and support the relevance and utility of retinal imaging for *in vivo* characterization of transgenic mouse model phenotypes as well as therapeutic evaluations.

Disclosures: **E.M. Rockenstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies. F. Consulting Fees (e.g., advisory boards); Neuropore Therapies. **D.L. Price:** A. Employment/Salary (full or part-time):; Neuropore Therapies. **D. Bonhaus:** A. Employment/Salary (full or part-time):; Neuropore Therapies. **M. Mante:** None. **J.D. Lindsey:** None. **E. Masliah:** 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.; Neuropore Therapies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.06/M1

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation (Syn TAP 2013)

NIH-AG18840 to EM

EVER Neuro Pharma

Title: The novel alpha-synuclein stabilizer NPT200-11 improves behavior, neuropathology, and Biochemistry in the murine thy1-ASYN transgenic model of Parkinson's disease

Authors: *M. A. KOIKE[1], D. L. PRICE[1], B. M. WHITE[1], E. ROCKENSTEIN[2], W. WRASIDLO[2], I. TSIGELNY[2], D. MEIER[1,3], E. MASLIAH[2], D. W. BONHAUS[1]
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Abstract: Parkinson's disease (PD) is characterized by aberrant accumulation of oligomeric forms of alpha-synuclein (ASYN). It is hypothesized that these toxic forms of ASYN contribute to the neuronal dysfunction and cell death observed in PD and other synucleinopathies, in part, though the formation of pore-like structures in cell membranes. We have developed a novel compound, NPT200-11, designed to ameliorate PD-related symptoms and pathology by selectively blocking the formation and accumulation of these toxic species of ASYN. We evaluated the activity of this compound in a transgenic mouse model of PD overexpressing human wild-type ASYN under the Thy-1 promoter, by administering NPT200-11 at 0, 1 or 5mg/kg (IP) once daily (5 days per week) for 3 months and then assessing PD-relevant sensorimotor performance, biochemical alterations and neuropathological changes in ASYN and related proteins. We used the Round Beam Task to assess sensorimotor impairments, using number of slips as our primary outcome measure, and found that transgenic mice treated with NPT200-11 at both doses (1 & 5mg/kg) had statistically significantly reductions in slips

compared to vehicle-treated transgenic mice. Western Blot analysis of cerebral cortical and hippocampal brain homogenates revealed statistically significant reductions in transgenic ASYN protein levels. Similarly, neuropathological assessment found a significant reduction in ASYN immunolabeling in the cortical neuropil of mice treated with NPT200-11 at both concentrations (1 & 5mg/kg). Moreover, we found normalization of neurodegeneration-related markers including NEUN and GFAP. Together, these results demonstrate that NPT200-11 significantly improves sensorimotor, biochemical and neuropathological outcomes in a transgenic mouse model.

Disclosures: **M.A. Koike:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. **D.L. Price:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. **B.M. White:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. **E. Rockenstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc. **W. Wrasidlo:** 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.; Neuropore Therapies Inc. **I. Tsigelny:** 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.; Neuropore Therapies Inc. **D. Meier:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc. **E. Masliah:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc. **D.W. Bonhaus:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies Inc..

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.07/M2

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation (Syn TAP 2013)

NIH-AG18840 to EM

EVER Neuro Pharma

Title: The novel alpha-synuclein stabilizer NPT200-11 reduces retinal deposits of ASYN-eGFP in a transgenic mouse model of Parkinson's disease/Lewy body disease

Authors: *D. L. PRICE[1], E. M. ROCKENSTEIN[2], M. MANTE[2], W. WRASIDLO[2,1], E. MASLIAH[2], D. W. BONHAUS[1], D. H. MEIER[1,3]
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Abstract: Abnormal accumulation of a neuronal protein alpha-synuclein (ASYN) has been hypothesized to underlie neuronal cell death and synaptic dysfunctional in Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Compounds that selectively interfere with alpha-synuclein protein-folding dynamics and prevent the formation of propagating dimers have been developed and further evaluated in animal models. One such compound, NPT200-11, has been shown to reverse motor deficits, and to normalize total alpha-synuclein levels and neurodegenerative markers in mThy1-ASYN transgenic mice overexpressing human ASYN. In addition to further characterizing the biological activity, safety and pharmacokinetics of NPT200-11 as a clinical candidate for the treatment of PD studies are underway to identify translatable secondary or surrogate markers for clinical evaluation. We previously demonstrated the feasibility of repeated longitudinal retinal imaging evaluations of e-GFP- ASYN in the PDNG78 transgenic mouse model of DLB/PD (Rockenstein et al., 2013), as a method to evaluate and track the progression of neurodegenerative changes in animal models of Parkinson's disease. Progressive pathological features in the PDNG78 retina were shown to mirror CNS pathology, thereby providing a means to non-invasively and repeatedly evaluate potential therapeutic interventions in a transgenic mouse model of PD/DLB. In the present study repeated longitudinal measures of eGFP-tagged ASYN in the retina demonstrated a robust, statistically significant time-dependent reduction in ASYN in the retina of NPT200-11-treated transgenic mice (5 mg/kg, IP, daily for up to 3 months).

Disclosures: **D.L. Price:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **E.M. Rockenstein:** F. Consulting Fees (e.g., advisory boards); Neuropore Therapies, Inc.. **M. Mante:** None. **W. Wrasidlo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **E. Masliah:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuropore Therapies, Inc. **D.W. Bonhaus:** A. Employment/Salary (full or part-time):; Neuropore Therapies, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified

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Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.08/M3

Topic: C.03. Parkinson's Disease

Support: Research Funding for Longevity Sciences (23-2 and 26-23) from National Center for Geriatrics and Gerontology (NCGG)

Title: Role of lipid peroxides derived from PUFA in the conformational change of alpha-synuclein and cell death in Parkinson disease

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Abstract: The pathological hallmark of Parkinson disease (PD) is the death of dopaminergic neurons in the substantia nigra and the intracellular accumulation of Lewy body and Lewy neurite, whose main component is the fibrillar form of alpha-synuclein (aSyn) with β-sheet structure. In the brain, polyunsaturated fatty acid (PUFA) is abundant and aSyn shows high affinity to the membrane enriched in PUFA chains and stabilizes conformation of αSyn in alpha-helical structure. Docosahexaenoic acid (DHA) is a major fatty acid composition of the neuronal membrane. DHA has been believed to be neuroprotective, by its anti-oxidative capacity and activating survival signaling. However, we found in rodents treated with CCl4 in addition to DHA, DHA-derived carboxylalkylamide adducts -modified proteins, were increased with marked necrosis of hepatic cells, but not in rodents fed with DHA-rich diet alone, or treated with CCl4 alone. In Parkinson disease, it is known that oxidative stress in increased in the dying neurons. Oxidation of membrane lipids, and modification of aSyn by ROS, RNS, and lipid radicals may decrease the affinity of aSyn to the membrane. In addition, oxidation of membrane-composing phospholipids should alter the physicochemical properties of the membrane, such as

membrane fluidity and permeability, and the fusion and fission of mitochondrion. Accumulation of aSyn with abnormal structure has been proposed to be associated with the pathogenesis of Parkinson disease. The cytotoxicity of αSyn depends on its higher structure, including momomeric, oligomeric and aggregated forms. This paper reports that DHA peroxidation modified αSyn and induced the oligomerization and facilitated the amyloidogenesis *in vitro*. In SH-SY5Y cells overexpressing aSyn, DHA induced cell death with increased aSyn adduct with N-acyl product from DHA peroxidation in the RIPA-soluble mitochondrial fraction and also in insoluble fraction. These results suggest that under oxidative stress, DHA-dependent modification of aSyn may initiate toxic ologomerization of aSyn even that in physiological condition, DHA functions as a lipophilic antioxidant. The biphasic function of DHA, either in a cytotoxic and protective way, is discussed in relation to the pathogenesis of PD.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson’s Disease

Support: Marie Curie Initial Training Network Grant Agreement: 215618

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ERA-Net NEURON

Title: Triggering mutant (a30p) alpha-synucleinopathy in mice

Authors: *S. NUBER[1,2,3], N. CASADEI[2], M. DIEPENBROEK[2], D. TADROS[1], A.-M. POEHLER[3], B. ETTLE[3], J. KLUCKEN[3], J. WINKLER[3], O. RIESS[2], E. MASLIAH[1,4]

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Abstract: Presence of Lewy bodies and neurites is a hallmark of Parkinson's disease. Occurrence of its main component alpha-synuclein as a fibrillized, ubiquitinated and truncated constituent suggests that impaired protein clearance is an important event in aggregate formation. The A30P mutation is known for its fast oligomerization, but slow fibrillization rate. Despite its toxicity to neurons, mechanisms involved in either clearance or conversion of A30P alpha-synuclein from its soluble state into fibrils and their effects *in vivo* are poorly understood. We therefore triggered animals overexpressing A30P alpha-synuclein either with environmental neurotoxic factors, or by genetic crossings known to implicate in alpha-synuclein degradation pathways and analyzed its impact on protein clearance and phenotype progression. We observed that calpain-cleavage, induced by paraquat exposure, led to an increase in C-terminal truncation and aggregation pathology. Vice versa, overexpression of the calpain-inhibitor, calpastatin, reduced truncation and aggregation pathology. Interestingly co-overexpression of C-terminal interacting protein synphilin-1 reduced alpha-synuclein truncation and fibrillization, displaying an increase in the formation of autophagic-susceptible aggresomes. However, the lack of fibril pathology yet persistence of motor dysfunction in aged mice, supports the idea that fibrillized alpha-synuclein deposits are not solely linked to the phenotype progression.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson’s Disease

Support: KAKENHI 25293124

KAKENHI 24102505

Title: FABP3 promotes α-synuclein oligomerization in the dopaminergic neurons

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Abstract: [Background] Fatty acid-binding protein 3 (FABP3) is highly expressed in the dopaminergic neurons, especially in the substantia nigra pars compacta (SNpc) (Shioda et al., J Neurosci, 2010;30:3146-55). FABP3 null mice exhibit hypo-dopaminergic phenotype in the extrapyramidal symptom. Notably, α-synuclein accumulation is critical to the pathogenesis of Parkinson's disease (PD) and is regulated by long-chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid (AA) and docosahexaenoic acid (DHA). We have recently hypothesized that FABP3 triggers α-synuclein accumulation in the dopaminergic neurons. [Methods] MPTP-induced α-synuclein oligomerization was assessed in wild and FABP3 null mice. *In vitro* studies evaluated the promotion of α-synuclein oligomerization by making complexes with FABP3. [Results] MPTP-induced α-synuclein oligomerization in the SNpc was attenuated in the FABP3 null mice compared to wild mice. Immunohistochemical analyses revealed that MPTP-induced α-synuclein accumulation in the SNpc was also attenuated in FABP3 null mice. *In vitro* study confirmed that α-synuclein oligomerization with FABP3 was promoted by AA (100μM) treatment. [Conclusion] Taken together, complex of FABP3 with AA is risk factor for the formation of α-synuclein accumulation in the dopaminergic neurons of PD patients. The evidences propose a novel therapeutic strategy for PD. [Acknowledgements] This work was supported by KAKENHI 25293124 and 24102505 (KF).

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Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

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Topic: C.03. Parkinson’s Disease

Support: Swedish Research Council

Swedish Brain Foundation

Swedish Alzheimer Foundation

Marianne and Marcus Wallenberg Foundation

Swedish Parkinson Foundation

Title: Characterization of the cellular uptake of α-synuclein oligomer/protofibril selective antibodies

Authors: *M. INGELSSON[1], G. GUSTAFSSON[1], F. ERIKSSON[2], E. NORDSTRÖM[2], C. MÖLLER[2], L. LANNFELT[1], J. BERGSTRÖM[1]
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Abstract: Background Immunotherapy targeting α-synuclein has emerged as a potential therapeutic strategy against Parkinson's disease and other disorders with Lewy body brain pathology. We have developed oligomer/protofibril selective antibodies [1] and shown that administration of such antibodies can lower the levels of toxic α-synuclein oligomers/protofibrils both in human cell lines [2] and in the spinal cord of transgenic mice [3]. However, it is not known whether these antibodies can be taken up by cells and whether they exert their anti-oligomer effects intra- or extracellularly. **Objective** To investigate whether oligomer/protofibril selective α-synuclein antibodies can be internalized by cells in culture. **Methods** Native or α-synuclein:hemi-GFP BiFC overexpressing H4 human neuroglioma cells were subjected to the monoclonal oligomer/protofibril selective α-synuclein antibodies mAb47, mAb49/G and mAb38E2 or to the non-selective α-synuclein antibodies Syn-1 and mAb211. An anti-GAPDH antibody was used as isotype control. All antibodies were incubated at 2.5 μg/ml for different time lengths in regular growth media at 37°C. Next, the cells were fixed followed by incubation with a fluorescent anti-mouse secondary antibody. The levels of remaining antibodies in conditioned media were measured by indirect ELISA. Double immunocytochemistry against α-synuclein and various organelle markers was carried out to assess the intracellular localization of the antibodies. **Results** All antibodies investigated were internalized by the cells. The oligomer/protofibril selective α-synuclein antibody mAb47 showed the highest degree of intracellular presence. The uptake was seen already after 1 hour and reached a maximum after 4 hours of incubation. Cells overexpressing α-synuclein displayed a higher antibody uptake than non-transfected cells. After 24 hours of incubation the antibody signal had almost completely vanished and the corresponding extracellular antibody levels had decreased, as measured by ELISA. **Conclusions** The oligomer/protofibril selective α-synuclein antibody mAb47 was readily internalized by cultured human neuroglioma cells. Ongoing studies will elucidate by which mechanisms this and other α-synuclein antibodies are taken up by the cells. **References** 1) Fagerqvist, T. et al. J Neurochem. 2013; 126(1):131-44 2) Näsström, T. et al. PLoS One 2011; 6(10):e27230 3) Lindström, V. et al. Neurobiol Dis 2014; *in press*

Disclosures: **M. Ingelsson:** F. Consulting Fees (e.g., advisory boards); BioArctic Neuroscience AB. **G. Gustafsson:** None. **F. Eriksson:** A. Employment/Salary (full or part-time):; BioArctic Neuroscience AB. **E. Nordström:** A. Employment/Salary (full or part-time):; BioArctic Neuroscience AB. **C. Möller:** A. Employment/Salary (full or part-time):; BioArctic Neuroscience AB. **L. Lannfelt:** E. Ownership Interest (stock, stock options, royalty, receipt of

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Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.12/M7

Topic: C.03. Parkinson's Disease

Support: the Strategic Research Program for Brain Sciences by the Ministry of Education

Title: Searching for a Lewy body in a mutated α-Synuclein (A30P) transgenic marmoset

Authors: *R. KOBAYASHI[1], C. HARA-MIYAUCHI[1,2], F. OZAWA[1], J. TAKAHASHI-FUJIGASAKI[3], J. OKAHARA[4], E. SASAKI[1,4], H. J. OKANO[1,2], H. OKANO[1]
[1]Keio Univ. Sch. of Med., Tokyo, Japan; [2]Div. of Regenerative Med., [3]Div. of Neuropathology, Jikei Univ. Sch. of Med., Tokyo, Japan; [4]Central Inst. for Exptl. Animals, Kanagawa, Japan

Abstract: Parkinson's Disease (PD) is the neurodegenerative disease with high frequency next to Alzheimer Disease. It is characterized by loss of dopaminergic neurons in a substantia nigra and the appearance of Lewy bodies, therefore PD is a kind of progressive malignant disease to cause muscle rigidity and tremor. In the brain, spinal cord and olfactory bulb, the Lewy body is composed of the protein α-Synuclein, phosphorylated α-Synuclein and also including other proteins. Abnormal aggregation of α-Synuclein and phosphorylated α-Synuclein is accepted at high frequency in neuronal cells of PD patients. It is suspected that abnormal accumulations of α-Synuclein trigger neurodegeneration. To clarify where the Lewy bodies and phosphorylated α-Synuclein appear, it is necessary to establish the non-human primate model. In our laboratory, a novel non-human primate model of PD using mutated α-Synuclein (A30P) was established to explore the effect of mutated α-Synuclein. As for the common marmoset (Callithrix jacchus), a small New World primate, its structure of the brain evolves in comparison with rodents. Therefore, we expect that it is possible to link between an exercise dysfunction and a lesion part because the structure of the basal nuclei which are a main lesion part of PD is clear in marmoset. The transgene of mutated α-Synuclein (A30P) expression was evident in the brain of transgenic marmoset, moreover we found the abnormal protein aggregations in the liver. Thus we searched for a Lewy body in the all tissues and organs of transgenic marmoset including skin. In this presentation, we will show the recent results of searching Lewy body and also the exercise functional analysis.

Disclosures: **R. Kobayashi:** None. **C. Hara-Miyauchi:** None. **F. Ozawa:** None. **J. Takahashi-Fujigasaki:** None. **J. Okahara:** None. **E. Sasaki:** None. **H.J. Okano:** None. **H. Okano:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.13/M8

Topic: C.03. Parkinson's Disease

Title: A53T mutation α-synuclein over-expression in rat brain show more toxic than wild-type via AAV injection

Authors: ***Y. DENG**, J. LU, H. QING, H. MA, R. WANG
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Abstract: There are many rodent models in Parkinson's disease, as 6-OHDA injection. AAV - mediated over-expression α-synuclein in rat brain is a newly developed technology in Parkinson's disease models. The gradually expression of α-synuclein in substantia nigra pars compact of rat after AAV injection accompany with loss of dopaminergic neurons is very similar to the clinical condition of Parkinson's disease. In this study, we used male wistar rats, and inject the AAV vectors which contain AAV-α-synuclein WT and its mutation A53T ,and EGFP to their substantia nigra pars compact by stereotaxic surgery, then keep them for 9weeks until take their brains. We found that when inject apomorphine i.p., the A53T group showed rotations but the other groups didn't show this. This data suggested that A53T α-synuclein is more toxic than WT. We use immunohistochemistry to identify that the AAV vectors expressed in substantia nigra and striatum. After that, the double- immunofluorescence method was used to find that the TH-positive cells were the cells which express α-synuclein/EGFP. Finally, the protease K digest method give us the result that A53T α-synuclein aggregate much more than WT. In summary, we establish a model to Parkinson's disease use AAV, and protein aggregation may play an important role in the cells loss progress. The mechanism of the A53T how to affect protein aggregation is still to be investigated.

Disclosures: **Y. Deng:** None. **J. Lu:** None. **H. Qing:** None. **H. Ma:** None. **R. Wang:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson's Disease

Support: Wallin Neuroscience Discovery Fund, NS038065

Title: Both soluble and insoluble α-synuclein species are capable of inducing pathology in the mouse model of α-synucleinopathy transmission

Authors: *J. BARNES, J. MEINTS, H. A. MARTELL-MARTINEZ, M. K. LEE
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Abstract: Pathological α-synuclein (αS) is implicated as a cause for neuronal dysfunction/neurodegeneration associated with Parkinson's disease (PD). Recent cell biological studies and extent human neuropathological studies suggest that αS pathology may spread from neuron to neuron. In particular, studies show that local injection of insoluble αS fibrils or crude brain lysates from tissues with severe α-synucleinopathy can induce global α-synucleinopathy in mouse brain. While previous work has explored possible mechanisms of *in vitro* neuronal αS release, uptake, and intracellular aggregation, little is known about such events *in vivo*. Specifically, the identity and toxicity of the transmissible αS species remain elusive. Moreover, the recently described intra-cortical/intra-striatal injection model of α-synucleinopathy demonstrates the spread of αS pathology in a direction counter to that proposed in humans. In order to establish a mouse model of αS transmission that more faithfully recapitulates the proposed spread in humans from the brain stem to anterior loci in the brain, we first established a model whereby αS pathology was initiated by injection of oligomeric/fibrillar forms of αS into the brain stem (dorsal motor nucleus of vagus, DMX) of young (3-4-month-old) mice overexpressing mutant HuA53TαS (line G2-3). Inoculation with *in vitro* pre-formed fibrils (PFFs) formed from recombinant αS or crude lysate (3000xg lysate) generated from end-stage HuA53TαS mouse tissue induced pathological αS alterations that spread in a highly reproducible spatial and temporal manner, reaching end-stage at ~70 days following inoculation. Because the crude brain lysates contain both soluble and insoluble αS species, the 3000xg lysate was further fractionated into highly soluble (S150) and insoluble (P150) fractions by centrifugation (150,000xg). Injection of S150 and P150 into the brain stem demonstrated that both fractions contain αS species sufficient to induce αS pathology that is subsequently transmitted anterogradely to the cortex. Conversely, neither saline nor 3000xg lysate from young asymptomatic HuA53TαS mice were capable of inducing αS pathology. Our results indicate that

distinct species of αS are sufficient to seed pathology in the brain stem that spreads in a predictable manner along known projection pathways in the brain. Further, this approach will allow us to identify the subcellular localization and biochemical nature of such toxic αS species.

Disclosures: J. Barnes: None. **J. Meints:** None. **H.A. Martell-Martinez:** None. **M.K. Lee:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: The Gardner Family Center for Parkinson's Disease and Movement Disorders

University of Cincinnati Neuroscience Institute

Network for Neuroscience Discovery

Title: The effect of dietary ketosis on mitochondrial function and alpha-synuclein accumulation in alpha-synuclein overexpressing mice

Authors: *A. M. LEHMKUHL[1], M. J. IRWIN[2], J. D. PANDYA[5], S. S. KARKARE[1], B. LIOU[6], R. KRIKORIAN[3], P. G. SULLIVAN[5], Y. SUN[6], S. M. FLEMING[4,1]
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Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by abnormal accumulation of the presynaptic protein alpha-synuclein (aSyn) and cell death. In addition to protein accumulation, mitochondrial impairment is well established in PD and thought to be an important contributor to cell loss. *In vitro* studies demonstrate an interaction between mitochondrial dysfunction and accumulation of aSyn, where alterations in either mitochondrial function or aSyn can lead to pathology and cell loss. It has been proposed that treatments that support mitochondrial function and/or reduce protein accumulation may have disease-modifying therapeutic potential in PD. Ketosis is a metabolic condition in which ketone bodies, as opposed to glucose, are utilized as the substrate for cerebral mitochondrial energy metabolism. The therapeutic potential of the ketone beta-hydroxybutyrate (BHB) on improving

mitochondrial bioenergetics is well documented. Additionally, BHB has been shown to stimulate lysosomal-autophagy degradation pathways in the liver, suggesting it may have therapeutic potential in PD by improving mitochondrial function and reducing aSyn accumulation. In the present study male wildtype (WT) and alpha-synuclein overexpressing (Thy1-aSyn) mice were fed either a control or ketogenic diet for 28 days starting at two months of age. Then, plasma BHB (*N*=21), mitochondrial bioenergetic parameters in the striatum (*N*=12), and soluble and insoluble aSyn in the ventral midbrain were measured (*N*=19). We found that while both WT and Thy1-aSyn mice fed a ketogenic diet showed increased plasma BHB levels compared to mice fed a control diet, Thy1-aSyn mice on the ketogenic diet exhibited a two-fold increase in plasma BHB levels compared to WT mice on the ketogenic diet. In addition, Thy1-aSyn mice fed a control diet show a reduced oxygen consumption rate compared to WT mice fed a control diet, while Thy1-aSyn mice on the ketogenic diet show significantly improved oxygen consumption and mitochondrial efficiency in the striatum compared to the Thy1-aSyn mice on the control diet. Soluble and insoluble aSyn protein levels were significantly increased in Thy1-aSyn mice fed the control diet compared to WT mice fed with the control diet. Taken together these findings suggest that by targeting mitochondrial bioenergetics as well as aSyn expression, BHB may have therapeutic, disease-modifying potential in PD.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson’s Disease

Support: Gift from Susan and David Plimpton, # NS038065

Title: Alpha-synucleinopathy in Transgenic Mouse Model is associated with impaired autophagy and lysosome function

Authors: *R. KARIM[1], M. K. LEE[2]
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Abstract: Abnormal α-synuclein (αS) accumulation in neurons, as Lewy bodies/neurites, is likely contributor to neurodegeneration in Parkinson's disease and other related disorders. While the cause of αS aggregation is unresolved, studies show that αS accumulation and autophagy/lysosomal system (ALS) to reciprocally regulate each other. Thus, aberrant accumulation αS may cause ALS defects and further exacerbation of αS pathology. As an initial test of this hypothesis, we asked whether the presence of αS pathology *in vivo* is linked to ALS defects. Analysis of transgenic (Tg) mouse model of α-synucleinopathy (*A53TαS*) reveal that the presence of αS pathology is associated with impaired ALS. Specifically, increase in autophagic marker protein, LC3-II, was associated with increase in the levels of p62, indicating to defect in autophagic flux. The defect in autophagic flux occurs despite the increase in lysosomal content as indicated by the increases in the lysosomal marker proteins, lamp-1 and cathepsin D. Collectively, our data indicate that α-synucleinopathy *in vivo* may lead to impaired fusion of autophagosome with lysosomes, leading to aberrant expansion of both compartments. We propose that this cascade of events promotes further progression of αS pathology and increases vulnerability of neurons to αS pathology.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.17/M12

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation for Parkinson's Disease

Title: Characterization of an α-synuclein rodent model of Parkinson's disease

Authors: *K. ALBERT[1], M. H. VOUTILAINEN[2], B. K. HARVEY[3], S. AHOLA[1], R. TUOMINEN[2], M. AIRAVAARA[1], M. SAARMA[1]
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Abstract: Background: In Parkinson's disease (PD), the core motor symptoms are tremor, rigidity, slowness of movement, and difficulty with posture. It principally occurs through degeneration of dopamine neurons in the substantia nigra (SN). There are various methods of inducing Parkinsonian-like symptoms in rodents, such as the toxins 6-OHDA or MPTP models,

however these do not necessarily properly characterize the human condition. α-synuclein (α-syn) is mutated in familial forms of PD, and it is a major component of insoluble protein aggregates called Lewy bodies. Therefore, α-syn is strongly considered to be one of the major causes of dopamine neuron degeneration. α-syn pathology can be induced in rodents through injection of human WT or mutant α-syn into the SN, where it induces neurodeneraration and upregulates ER stress. This contributes to dysregulation of the unfolded protein response (UPR), and since α-synucleiopathies at least partially result from the misfolding of proteins, ER stress, UPR and their relation to α-syn are also important in PD and models of the disease. Objective: To create a rodent model of α-syn-induced PD that shows both α-syn overexpression as well as behavioral and molecular deficits for testing therapeutic efficacy of novel neurotrophic factors CDNF and MANF and mutants of GDNF family ligands. Methods: Stereotaxic injection of adeno-associated virus (AAV) vector carrying cDNA for human WT- α -syn or GFP in to one or two sites in the SN of rats. Both male Wistar rats as well as female SD rats were used, and tried commercial injection needles of different sizes as well as self-made coated glass capillaries. Amphetamine-induced rotations (2.5mg/kg, s.c.) and cylinder test were performed as behavioral assays. Immunohistochemistry of GFP, TH+ cells and α-syn+ cells in striatum (STR) and SN of the brains. Results: The behavior of male Wistar rats were followed for 5 to 7 months. We observed behavioral deficits only in the AAV-GFP injected animals. There was no difference in neurodegeneration between GFP and α-syn injected animals using a very thin, commercial needle, and a slow flow rate. In female SD rats, α-syn staining was found in SN after two sites of injections with commercial injection needles as well as by using glass capillaries. After 4 and 8 weeks, there is evidence of α-syn+ cells in both the STR and SN, and there is a decrease in TH fiber density in the STR. In both α-syn and GFP groups neurodegeneration was observed.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.18/N1

Topic: C.03. Parkinson’s Disease

Title: Behavioral, gastro-intestinal and histopathological findings in A53T α-synuclein mouse model of Parkinson's disease after LPS exposure

Authors: J. OKSMAN[1], C. BUENSUCESO[2], M. CERRADA-GIMENEZ[1], *A. J. NURMI[1], T. HUHTALA[1], J. HARRIS[2], U. HERZBERG[2]
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Abstract: Multiple commonly used transgenic animal lines exist for Parkinson's Disease (PD), with various approximations of an ideal Parkinsonian phenotype. However, success in creating a genetically altered animal line that shows a PD-like phenotype with robust and stable behavioral motor impairment, as well pathological tissue changes affecting the dopaminergic system, has proven to be a challenge. Also, many of the models do not express non-motor changes associated with PD, or they have not been investigated thoroughly. However, there is evidence that PD also affects various non-motor related changes in clinical disease, including disturbances in colon motility and fecal output. Targeted over-expression of human α-synuclein in a mouse model shows only a mild PD phenotype, even at 12 months of age. Therefore, we attempted to approach the disease model by using additional LPS challenges to enhance disease progression, and to assess by behavioral monitoring and nuclear imaging. In addition, we attempted to monitor colon motility as a non-motor endpoint over time. Twelve month-old A53T transgenic mice and corresponding wild type mice were tested for baseline behavior followed by LPS challenge over 3 consecutive weeks. During LPS injections and after a 16 week follow-up period, behavioral monitoring by beam balance test and beam traversing were performed. In addition, non-motor effects of the A53T transgene as well as additional challenge by LPS were monitored by a fecal output assay. Peripheral TSPO ligand (CLINDE) binding activity, which is an inflammatory marker, was analyzed by SPECT/CT imaging over time. Finally, at the endpoint, brains were collected and processed for dopamine and its metabolites from the striatum and histological evaluation of tyrosine hydroxylase and GAP 43 positive cells from substantia nigra (SN). Furthermore, expression of α-synuclein was monitored and evaluated between non-challenged and LPS-challenged A53T mice. This study focused on the validation of a previously published mouse model of PD in mice with novel endpoints and additional challenges. We present data about the progression of the PD phenotype up to 16 months of age, the effect of LPS on the phenotype, and whether biochemical, histopathological, and nuclear imaging markers correlate with behavioral changes.

Disclosures: J. Oksman: None. **A.J. Nurmi:** None. **M. Cerrada-Gimenez:** None. **T. Huhtala:** None. **C. Buensuceso:** None. **J. Harris:** None. **U. Herzberg:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.19/N2

Topic: C.03. Parkinson's Disease

Support: Georgetown University

Title: Tyrosine kinase inhibition regulates early systemic immune changes and modulates the neuroimmune response in α-Synucleinopathy

Authors: ***M. HEBRON**, I. LONSKAYA, S. SELBY, C. MOUSSA, F. PAGAN
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Abstract: Objectives. Neuro-inflammation is common in α-Synucleinopathies and Tauopathies; and evidence suggests a link between the tyrosine kinase Abl and neurodegeneration. Abl upregulates α-Synuclein and promotes Tau hyper-phosphorylation (p-Tau), while Abl inhibitors facilitate autophagic clearance. Methods. A model of α-Synucleinopathy harboring human mutant A53T α-Synuclein and exhibits concomitant increase in murine p-Tau was used to determine the immunological response to Abl inhibition. Results. Age-dependent alterations of brain immunity, including loss of IL-10 and decreased levels of IL-2 and IL-3 were observed in old A53T mice. Brain CCL2 and CCL5 were decreased, but CX3CL1 remained constantly elevated. Young A53T mice exhibited differential systemic and central immune profiles in parallel with increased blood markers of adaptive immunity, suggesting an early systemic immune response. Tyrosine kinase inhibitors (TKIs), including nilotinib and bosutinib degraded brain and peripheral α-Synuclein and p-Tau and modulated blood immunological responses. TKIs did not affect brain IL-10, but they changed the levels of all measured blood immune markers, except CX3CL1. TKIs altered microglia morphology and reduced the number of astrocyte and dendritic cells, suggesting beneficial regulation of microglia. Conclusions. These data indicate that tyrosine kinase inhibition affects neuro-inflammation via early changes of peripheral immune profile, leading to modulation of neuro-immune response to α-Synuclein and p-Tau.

Disclosures: **M. Hebron:** None. **I. Lonskaya:** None. **S. Selby:** None. **C. Moussa:** None. **F. Pagan:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 411.20/N3

Topic: C.03. Parkinson's Disease

Title: Alterations in stimulation-evoked dopamine release and associated behaviors in mice lacking GSTpi and transgenic mice carrying the A53T alpha-synuclein mutation

Authors: *D. B. LESTER, K. J. SAMPLE, R. J. SMEYNE
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Abstract: Alpha-synuclein is a major constituent of Lewy bodies, the pathognomonic protein aggregate seen in both familial and sporadic Parkinson's disease (PD). Clinically, the A53T mutation within the alpha-synuclein gene results in dominantly inherited PD, thus we have made an effort to understand the pathophysiology of this mutation using transgenic mice. Previously we have shown that transgenic A53T mice have a slightly reduced number of SNpc dopaminergic neurons, but no alterations in basal dopamine (DA) content at 12 months old. Since PD patients with A53T alpha-synuclein polymorphisms show wide variations in symptom manifestation and severity, it has been suggested that other biological processes contribute to the detrimental effects of the mutation. One such process is oxidative stress. Previously we have shown that genetic deletion of the antioxidant protein glutathione S-transferase pi (GSTpi) in mice increases the sensitivity of SNpc dopaminergic neurons to exogenous parkinsonian toxins. Therefore, we set out to determine if loss of GSTpi could exacerbate the pathology of these A53T mice. However, as previously reported, loss of GSTpi had no effect on SNpc dopaminergic cell number, striatal DA content, or time on the rotarod in WT or A53T mice. To further analyze the neurochemical properties of the nigrostriatal DA system in these strains, the present study utilized *in vivo* fast scan cyclic voltammetry (FSCV) with carbon-fiber recording microelectrodes in anesthetized mice (12 months old) to measure changes in striatal DA release evoked by brief electrical stimulation of the medial forebrain bundle. GSTpi-/- mice showed reduced concentration of stimulated DA release as well as reduced DA half-life compared to WT mice. Loss of GSTpi did not reduce these properties in transgenic A53T mice; however, A53T mice already displayed reduced stimulated DA release and DA half-life. Administration of the DA transporter (DAT) blocker nomifensine (10mg/kg, ip) increased the DA half-life of GSTpi-/- mice (279%) to a significantly greater degree than that of the WT (208%), which may explain the large increase in locomotor activity observed in GSTpi-/- mice following nomifensine. Although loss of GSTpi does not alter the DA systems of A53T mice to the same extent, the measured properties (stimulated DA release, DA half-life, and locomotor activitiy following nomifensine) of A53T mice more closely resemble those of GSTpi-/- mice rather than the WT. The mechanism underlying this physiological change is unknown but may be related to differences in DAT densities or available DA supply. Further studies will be conducted to look at these possibilities as well as the impact of aging.

Disclosures: D.B. Lester: None. **K.J. Sample:** None. **R.J. Smeyne:** None.

Poster

411. Parkinson's Disease: Alpha-Synuclein

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Topic: C.03. Parkinson's Disease

Support: NIH grant (DA017279, AG033954, DA037161, MH086383),

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The Caltech Innovation Initiative

The Caltech Millard and Muriel Jacobs Genetics and Genomics Laboratory.

Title: Transcriptional regulation by nicotine or α-synuclein in dopaminergic neurons assessed by few-cell and single-cell RNA-Seq

Authors: *B. M. HENLEY[1], B. A. WILLIAMS[2], R. SRINIVASAN[2], B. N. COHEN[2], C. XIAO[2], E. D. W. MACKEY[2], F. RICHTER[3], P. DESHPANDE[2], S. MCKINNEY[2], M.-F. CHESSELET[3], B. J. WOLD[2], H. A. LESTER[2]
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Abstract: Dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) degenerate in Parkinson's disease (PD). These neurons robustly express several nicotinic acetylcholine receptor (nAChR) subtypes. Smoking appears to produce neuroprotection against Parkinson's disease but the mechanism is unknown. To determine whether nicotine-induced changes in gene expression that contribute to the neuroprotective effects of smoking, we developed methods to measure the effect of prolonged nicotine exposure on the SNc neuronal transcriptome in an unbiased manner. Pools of twenty neurons were collected using laser-capture microscopy, and transcriptional changes were assessed using RNA deep sequencing. Overall, the expression of 129 genes was significantly affected by chronic nicotine, including genes involved in the ubiquitin-proteasome pathway, cell cycle regulation, chromatin modification, DNA binding, and RNA regulation. Using the same approach and an α- synuclein overexpression (Thy1-aSyn) mouse model, we measured the effects of α-synuclein expression on the SNc neuronal transcriptome. Our data show that DA neurons isolated from the α-synuclein Parkinson's disease mouse model display striking dysfunction of mitochondrial gene expression and have higher levels of oxidative stress. We also report preliminary transcriptome data for single-cell dopaminergic and GABAergic neurons isolated from midbrain cultures. In summary the results

show that nicotine can modulate expression of numerous genes in several pathways. The pathways modulated by nicotine are relevant to Parkinson's disease and may participate in the neuroprotective effects of smoking. Additionally, we show that over-expression of α-synuclein causes marked cellular stress relevant to PD within the DA neurons of the SNc. These novel techniques will facilitate advances in understanding the mechanisms taking place at the cellular level and will have applications elsewhere in the fields of neuroscience and molecular biology.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Program#/Poster#: 411.22/N5

Topic: C.03. Parkinson's Disease

Support: Morris K. Udall Center of Excellence for Parkinson's disease Research at Michigan State University

Title: Evaluation of the effects of aging in an induced alpha-synucleopathy rat model

Authors: I. M. SANDOVAL[1], K. C. LUK[2], S. CELANO[1], N. L. MARCKINI[1], B. F. DALEY[1], J. Q. TROJANOWSKI[2], V. M. LEE[2], K. L. PAUMIER[1], *T. J. COLLIER[1]
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Abstract: Previous studies demonstrate that injections of pre-formed fibrillar (PFF) forms of alpha-synuclein (α-syn) into the mouse striatum can induce Parkinson's-like accumulation of aggregates in anatomically interconnected regions, which results in significant neurodegeneration. Our laboratory has replicated this finding in a rat, thereby inducing a progressive, neurodegenerative rat model of α-synucleinopathy. This model exhibits hyper-phosphorylated α-syn intraneuronal accumulations (i.e., diffuse Lewy neurite (LN)- and LB-like inclusions) as early as 30 days in several areas interconnected with the striatum, most prominently in the frontal and piriform cortices and the substantia nigra (SN). Furthermore, α-syn pathology in this model co-localizes with ubiquitin, indicating they share common properties with human LBs/LNs. While this model was established in young animals, one of the major risk

factors for PD is aging; therefore, we examined the effect of PFFs in aged animals. We are specifically interested in whether age influences the localization and accumulation of α-syn to the nucleus, which may increase the vulnerability of dopamine neurons. Additionally, we expect that age may impact the rate of α-syn propagation since reduced transport and protein synthesis are known to be impaired in older animals. To this end, young (2 month) and old (20 month) male Fischer rats received a single intrastriatal injection of either 8 or 12ug of mouse PFFs. Quantitative analysis of intraneuronal accumulations and dopamine degeneration within the SN of rats will determine whether aging impacts α-syn propagation and dopamine neuron vulnerability. Preliminary analysis of brain sections collected 60 days post-injection reveals substantially less LB-like inclusions in the aged rats compared to young rats, suggesting a less efficient spread of α-syn fibrils in aged brains. Stereological analyses are underway to determine whether the aged brain is more susceptible to α-syn-associated toxicity, resulting in more pronounced degeneration of SN dopamine neurons. Results from these studies will shed light on whether pathological forms of α-syn behave differently in an aged brain and provide further evidence that normal aging is an underappreciated factor in neurodegenerative disease.

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Poster

411. Parkinson's Disease: Alpha-Synuclein

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Program#/Poster#: 411.23/N6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS083410

Title: Glia, glutamate receptors and oligomeric alpha-synuclein

Authors: *E. DAVIS, K. CRADDOCK, K. CONANT, K. MAGUIRE-ZEISS
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Abstract: The accumulation of oligomeric alpha-synuclein is a hallmark feature of a group of neurologic disorders called synucleinopathies. In addition to Parkinson’s disease, multiple system atrophy and diffuse Lewy body disease, synuclein is also evident in the substantia nigra of HIV-infected individuals. Glial activation is also present at both early and late stages of these

disorders, suggesting a role for glia in the progression of disease. Previously we demonstrated that oligomeric alpha-synuclein directly activates microglia leading to a complex inflammatory response via a toll-like receptor mediated pathway. Interestingly, monomeric alpha-synuclein did not incite a microglial inflammatory response, demonstrating a role for protein structure. Here we further characterize this glial activation by investigating the effect of oligomeric synuclein on glutamate receptors. Since glutamate receptor composition can modulate intracellular calcium levels, oligomeric synuclein-mediated alterations in these receptors would impact overall glial function. Here we also characterize the effect of oligomeric synuclein on astrocyte function; a glial cell type critical for calcium regulation. These studies investigate a novel role for oligomeric synuclein-induced glial activation.

Disclosures: **E. Davis:** None. **K. Craddock:** None. **K. Conant:** None. **K. Maguire-Zeiss:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS077657-02

Title: Pallidal deep brain stimulation in cortical-cortical and cortical-subcortical structures in the behaving 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine nonhuman primate hemi-Parkinson’s disease model

Authors: *C. M. HENDRIX, A. MURALIDHARAN, K. BAKER, J. VITEK
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Abstract: Current hypotheses propose that therapeutic deep brain stimulation (DBS) improves motor signs in Parkinson’s disease (PD) by modifying abnormal patterns of neuronal activity in subcortical-cortical and cortical-cortical motor circuits. How motor improvements through therapeutic DBS correlates with the changes in neuronal activity remains unclear. This study examines this relationship through concurrent cortical-cortical single unit (SU) recordings in the premotor (PM), primary motor (M1), and supplemental motor (SMA) areas in the hemi-Parkinsonian 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate undergoing pallidal DBS while performing a cued, center-out reaching task. Pallidal DBS

improved reaction times and reduced variability in reach behavior and periods of freezing. Beta and gamma band synchronization was more closely time locked to the task related go cue during DBS. Beta band desynchronization was time locked to reach onset under both DBS on/off conditions. Cortical SU peristimulus time histograms indicated a general inhibition of cortical activity while perievent time histograms aligned on task behaviors indicated an increase in the modulation of activity across epochs during DBS. Preparatory activity in SMA cells was present during DBS compared to little or no activity in the same cells while off-DBS. During DBS, peak coherence was more prevalent in the beta band for SUs across SMA-M1/PM structure while theta to alpha and gamma bands were more likely to appear within each cortical structure. Reduction of beta band activity has been linked to the development of bradykinesia. However, it is more likely that beta band activity is necessary and integral to normal movement; pallidal DBS acts to change the timing of such activity during motor behavior by facilitating time-locked beta band synchronization within the structure. An increase in time-locked modulation with DBS was also noted in/across gamma and theta-to-alpha bands suggesting that improvement in behavior may occur as the result of a combination of temporally correlated time locked changes across multiple power spectrums. DBS related inhibition and pattern changes in cortical activity with corresponding improvement in motor behavior is consistent with the hypothesis that pallidal DBS improves motor behavior through the suppression of non-selective firing patterns (noise), changes in selective firing patterns, and the temporal relationships of neural activity across frequency bands within the thalamo-cortical motor pathways.

Disclosures: C.M. Hendrix: None. **J. Vitek:** None. **K. Baker:** None. **A. Muralidharan:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson’s Disease

Title: First evidence of neural mechanisms of Hebbian changes in white matter tracts induced by long-term deep brain stimulation

Authors: *T. J. VAN HARTEVELT[1,2], J. CABRAL[1,3], A. MØLLER[2], J. J. FITZGERALD[5], A. L. GREEN[5], T. Z. AZIZ[5], G. DECO[3,4], M. L. KRINGELBACH[1,2,5]
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Catalana de la Recerca i Estudis Avançats, Univ. Pompeu Fabra, Barcelona, Spain; [5]Nuffield Dept. of Surgical Sci., John Radcliffe Hosp., Oxford, United Kingdom

Abstract: Hebbian learning has not been demonstrated at the macroscopic scale of long-range connections in humans, although behavioural changes from deep brain stimulation (DBS) suggest structural changes. It is not clear, however, which of the many connections from a given DBS target are most influential in providing the clinical benefit and if DBS creates long-term changes in brain connectivity. We used the rare opportunity of having preoperative and five-month postoperative diffusion tensor imaging (DTI) data from a patient with DBS of the subthalamic nucleus (STN) for Parkinson's disease which allowed us to reconstruct the spontaneous dynamics of the underlying pre and post connectivity measures. We used a whole-brain computational model to explain DBS-induced Hebbian long-term structural changes using simulated functional connectivity based on the pre- and postoperative DTI data. We calculated the difference between the pre-DBS and post-DBS structural connectivity (SC) and between the simulated functional connectivity (FC) matrices. The post-DBS FC matrix was calculated based on the pre-DBS SC matrix with a stimulation input in the STN included to simulate the DBS effects. The difference of the simulated functional and empirical structural connectivity matrices was compared to find the optimal stimulation factor through Hebbian learning. This stimulation factor was based on optimising the weights of the known connections from the STN so that the FC difference best fits the SC difference. Using rare pre- and post-DBS DTI data from Parkinson's disease, we showed significant changes in structural connectivity which led to significant functional alterations. Using a whole-brain computational model we showed that DBS appears to have its effect through changing the connectivity weights of the putamen and the thalamus, but not the caudate or STN. These functional changes led to clinical improvement as well as structural changes that improved towards normality. This is the first study providing evidence that DBS changes the functional weights of selective connectivity from the implanted electrode in the STN. This is highly suggestive of neural Hebbian changes in white matter tracts induced by long-term DBS. This novel approach allows us to determine the relative contributions of the connectivity of the DBS target and to track any long-term Hebbian changes induced by DBS.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson's Disease

Support: St. Jude Medical

Title: Randomized burst patterns of stimulation produce long-lasting, dose-dependent improvement in bradykinesia in the parkinsonian non-human primate

Authors: *J. WANG[1], A. MURALIDHARAN[1], L. VENKATESAN[2], C. VETRUBA[1], J. VITEK[1], K. BAKER[1]
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Abstract: Unconventional deep brain stimulation (DBS) paradigms are being explored for their potential to enhance therapeutic efficacy in patients with Parkinson's disease (PD). This includes stimulus patterns that may induce beneficial effects that outlast stimulus delivery, as previously demonstrated for akinesia by Tass and colleagues. The current study examined further the potential for randomized burst (RB) DBS patterns to mitigate parkinsonian motor signs as a function of dose duration using the MPTP monkey model of PD. Two rhesus macaque primates were operantly trained to allow passive limb manipulation and to perform a standard reaching task. Each was implanted with a scaled-down DBS lead in the STN region. A repeated-measures, block design was applied to evaluate and compare the effects of isochronal (2 hours/day; 130 Hz; 0.55 mA) DBS to RB stimulation, with the latter delivered for 2- (RB2) or 4-hours (RB4) per day at 0.2 mA. A chronically implanted Brio™ IPG (St. Jude Medical, Plano TX) with research software was used to randomly deliver bursts of stimulation to each of the three ventral-most contacts, with the most dorsal contact programmed as the anode. Each paradigm was applied for five days, followed by a DBS OFF block to characterize carry-over effects. Reaching data and modified UPDRS (mUPDRS) scores were collected daily, with the duration of each post-treatment, DBS OFF block extended until the mUPDRS score returned to baseline. mUPDRS scores and reach/retrieval velocity measures were improved by isochronal, RB2 and RB4 DBS, with therapeutic carryover observed for up to 3, 5, and 14 days, respectively. Post-DBS improvements of mUPDRS associated with isochronal and RB2 DBS were as high as 15%, while RB4 DBS yielded improvements of up to 29%. Reaching task behavior was marked by increased velocity at both the wrist and elbow, with post-DBS improvements at the wrist of up to 25%, 7% and 26%, and the elbow of up to 23%, 6%, and 43% for isochronal, RB2, and RB4 DBS, respectively. Our data are consistent with previous studies suggesting that both acute and long-term therapeutic benefit can be achieved using unconventional DBS patterns. Uniquely, our data suggest that carry-over of therapeutic benefit following discontinuation of DBS may be sensitive to dose duration, with enhancement of both the magnitude and endurance of after-effects achieved by increasing the dosing window. Moreover, these results underscore the need for further studies to understand the mechanism(s) underlying this therapeutic effect as a

potential means of deriving rationale for the development of novel stimulation paradigms to optimize further the magnitude and duration of therapeutic benefit.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.04/N10

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS037019-14

Title: Developing closed-loop control of deep brain stimulation using oscillations in local field potentials

Authors: ***L. A. JOHNSON**[1], E. PARK[2], C. M. HENDRIX[1], K. B. BAKER[1], J. L. VITEK[1]
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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) or the globus pallidus internus (GPi) is an effective treatment option for many individuals with advanced Parkinson's disease (PD). Although patient's clinical symptoms may vary throughout the day, contemporary DBS systems deliver stimulation in a continuous fashion without regard for the patient's clinical state. A promising approach to improve DBS therapy is to modify stimulation parameters in real time based on measures of brain activity. In this study, we developed a closed-loop recording/stimulation system that extracts oscillatory activity from local field potentials (LFPs) recorded differentially from two DBS contacts to trigger stimulation at an adjacent contact. The system allowed for flexible, user-defined control of recording, real-time analysis, and stimulation parameters. For initial testing, we used the amplitude of beta oscillations (13-35Hz) to trigger DBS, as STN and GPi LFP beta power has been shown to be a biomarker of disease in PD patients and the non-human primate MPTP model of PD. The closed-loop system was first tested in-vitro to determine if stimulation artifacts would corrupt the signal of interest. A voltage waveform generator was used to create sinusoidal signals with amplitudes the same order of magnitude as those observed in-vivo at frequencies ranging from 10 to 40 Hz. Spectral analysis

confirmed that the power at each of the frequencies evaluated was unchanged between DBS in the ON (130Hz) and OFF conditions. The closed-loop system was also tested in-vivo, in a rhesus macaque rendered parkinsonian through intra-carotid injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and implanted in GPi with an 8-contact DBS lead. The DBS trigger threshold was established based on baseline beta amplitude measured in the resting DBS-off condition. Threshold was systematically varied (25, 50, 75 percentile of baseline); stimulation continued as long as the beta amplitude was above the trigger level. The behavioral effects of closed-loop DBS were assessed while the animal performed a center-out reach task and compared to open-loop (isochronal, 130Hz) and DBS-off conditions. In this study we developed a platform to evaluate real-time, closed-loop DBS in an awake, behaving animal model of PD, which will be useful to investigate the optimal parameters for feedback control of DBS and to understand the neural mechanisms underlying the therapeutic benefit of DBS.

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Poster

412. Parkinson's Disease: Clinical Therapies

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NSF-GRFP-00006595

Title: Changes in the phase-amplitude coupling of high-frequency oscillations as a biomarker of parkinsonian severity

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Abstract: Background: Previous studies have associated the presence of beta oscillations (10-35 Hz) in basal ganglia local field potentials (LFP) with Parkinson's disease (PD), but few have correlated the role of high-frequency oscillations (HFO, >100 Hz) to the severity of parkinsonian motor signs (Özkurt et al., 2011). Objective: To study changes in LFP phase-amplitude coupling associated with parkinsonian severity in a non-human primate model of PD. Methods: Spontaneous LFPs were recorded from three awake NHPs as a bipolar signal between two microelectrodes located in the globus pallidus interna or externa. Recordings were made pre-MPTP exposure as well as at each of three consecutive parkinsonian states (mild, moderate and severe) achieved through successive MPTP injections. Motor severity was indexed using a modified UPDRS. Each trial was labeled according to parkinsonian state and recording location and filtered into frequency bands ranging from 0.1-390 Hz. The phase-amplitude coupling (PAC) across bands was assessed according to a modulation index (MI) and compared to a set of 100 time-shuffled bootstrapped surrogates. PAC was considered significant if the MI was different from the noise floor with a $Z>3$. Results: In the naïve state, significant PAC was observed in 61% (202/333)(of recordings between the gamma phase (32-128 Hz) and the HFO amplitude (128-390 Hz). As parkinsonian symptoms worsened, the proportion of recordings with significant PAC in these bands remained constant 61% (101/165)in mild, 68% (126/184)in moderate, and 62% (68/109)in the severe state, Fisher's exact $p=0.2166$). In contrast, the HFO amplitude became significantly coupled with beta band phase (8-32 Hz) as severity increased 16% (53/333) in naïve, 39% (65/165)in mild, 42% (77/184) in moderate, and 59% (64/109)in the severe state, Fisher's exact $p<0.001$). Conclusions: Coupling between the phase of beta oscillations and the amplitude of high frequency oscillations in the globus pallidus was found to correlate with increasing severity of motor signs in the non-human primate model of PD. These data suggest that abnormal synchronization may impair normally independent neural processes through the basal ganglia necessary for normal movement.

Disclosures: E.M. Bello: None. **A.T. Connolly:** None. **K.B. Baker:** None. **T.I. Netoff:** None. **M.D. Johnson:** None. **J.L. Vitek:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.06/N12

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS037019

Title: Effect of STN DBS on motor cortical activity in the resting state and during movement

Authors: A. MURALIDHARAN[1], J. ZHANG[1], F. AGNESI[2], S. NEBECK[3], *K. B. BAKER[1], J. L. VITEK[1]
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Abstract: Introduction: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been demonstrated to improve the cardinal motor signs of Parkinson's disease (PD). The relationship of these improvements to changes in motor cortical activity has not yet been characterized. In order to correlate the therapeutic effects of STN DBS on movement kinematics, changes in the activity and receptive field characteristics of neurons in the arm area of the motor cortex (M1) during passive-joint manipulation and a cued-reaching task were analyzed in this study. Methods: One female rhesus macaque was made parkinsonian by injecting the neurotoxin MPTP. The STN was mapped using microelectrode techniques and a scaled-down version of the human quadripolar-DBS lead was implanted in the right STN. Therapeutic stimulation parameters were assessed through behavioral testing and clinical examination. The effect of therapeutic DBS was determined by comparing single unit cortical activity before and during DBS while the animal was at rest, during passive manipulation of the joint corresponding to the unit's receptive field and the cued-movement task. Results: In the resting state, 54% of the neurons in the motor cortex were suppressed, 21% were activated and 12% showed no change during DBS. Sixty-seven per cent of the cells inhibited by DBS showed a tonic suppression, while a smaller percent (33%) expressed a bi-or poly- phasic discharge pattern. All of the units that were excited by DBS had bi- or poly- phasic response patterns. The distribution of DBS response types during passive-joint movement was similar to that seen at rest. Twenty-nine per cent of the cells were found to code for position, 24% to velocity and 46% to both in the pre-DBS interval. During DBS 28% changed their tuning characteristics, 32% lost tuning and 20% that did not respond to position or velocity were now tuned to one or both. In all the cells that exhibited responses to passive movements directional specificity was increased. During the cued-movement task, DBS increased the signal to noise ratio and rate of rise of neuronal activity. Conclusion: STN DBS affects multiple aspects of spontaneous and movement related activity in the motor cortex that occurs coincident with improvement in motor behavior. DBS appears to have a significant effect on information processing in the motor cortex reflected as an increase in the signal to noise ratio and directional specificity of M1 neurons during movement. The exact relationship between the improvement in motor control and associated changes in M1 neuronal activity (rate, pattern, directionality) remains unclear.

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Poster

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Program#/Poster#: 412.07/O1

Topic: C.03. Parkinson's Disease

Support: FOODCAST, Regione Lombardia, ISMEA

Title: A possible role of reward sensitivity and impulsivity in weight gain after deep brain stimulation

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Abstract: Introduction: Deep brain stimulation of the subthalamic nucleus (STN-DBS) has acquired a relevant role in the treatment of Parkinson's disease (PD). Despite being a safe procedure, it may expose patients to an increased risk to experience non-motor effects. For instance, many studies have shown that after the implant, patients with PD suddenly increase in weight. However, the exact mechanism and the predisposing factors of this weight gain have not yet been identified. Objective: Since these aspects have been demonstrated to be modulated by STN stimulation surgery, we aimed at evaluating the role of reward sensitivity and impulsivity in weight gain after DBS. Methods: Patients with PD scheduled for undergoing STN-DBS were recruited. They were tested before surgery in an on medication condition and after surgery in an on medication/on stimulation condition, in a satiated state. All participants were asked to perform a self-report questionnaire about impulsiveness, a go-no-go experiment measuring the response inhibition to food items and finally, a task that evaluates both hedonic and motivational aspects of food processing (liking and wanting, respectively). Food stimuli varied across two dimensions: taste (sweet vs. non-sweet) and calorie density (high vs. low). The preoperative and postoperative body weights were recorded. Results: The mean body weight of patients increased post-operatively. After STN-DBS, patients exhibited higher level of impulsivity. The weight changes correlated positively with impulsivity, hedonic ratings of food items (liking) and salience/motivation towards food (wanting) in the on medication/on stimulation condition whether no correlations were found with tastiness ratings of food. Moreover, strong correlations were observed between weight changes and liking for high calorie density food (both sweet and non-sweet) in the on medication condition before surgery. Conclusion: Our results confirm that STN-DBS may expose patients to the risk of weight gain. They also suggest that both

impulsivity and higher sensitivity to reward may play a significant role in the regulation of food intake and weight after surgery.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.08/O2

Topic: C.03. Parkinson's Disease

Support: Canadian Institute of Health Research - CGS

Title: Deep brain stimulation: A new approach to programming for Parkinson's disease

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Abstract: Objective: Out with the old and in with the new, technology is rapidly changing and it is essential clinical assessment tools keep pace. Deep brain stimulation (DBS) is a new treatment option provided to L-DOPA responsive Parkinson's disease (PD) patients who are not adequately controlled by their medication. DBS patients receive clinical scale based programming sessions for treatment of their gait. However this clinical scale based assessment for gait has shown inconsistent results. An objective, quantitative, assessment of DBS on gait has not been conducted. Our group uses a gait analysis carpet to track and assess the changes in gait following DBS parameter changes. Method: Three PD patients (age: 65±3.3, PD duration: 12.2±3.3) were recruited. All patients were eligible for STN-DBS procedure. The patients were assessed at five time points, once before surgery and four times post-surgery (at 1 month intervals). For the assessments the Unified Parkinsons Disease Rating Scale (UPDRS) and a gait capture carpet, using PKMAS software, were used. The patients performed Timed Up and Go task at both normal and fast speeds. Gait was assessed using functional ambulation profile (FAP) and stride length. The FAP score rates a patient's walk to a standardized data base (95-100 being normal). Results: The gait score (UPDRS Part III - Item 29) showed a fairly unchanged rating across visits (0.3, 0.3, 0.6, 1.0 and 0.3 on average), while the objective gait capture carpet tracked changes. The gait capture carpet showed a decrease in FAP scores from visit 1 to visit 5.

The changes in FAP scores were: patient E.V.: from 86.5 to 84.5 and patient L.M.: from 88.5 to 86.5. There was an improvement in FAP scores for patient F.Z.: from 73 to 78.5. Furthermore the stride length showed worsening in the patients (E.V.: from 102cm to 94.5cm; L.M.: from 105 to 100cm), and improvement in patient F.Z. (from 79.5 to 87). Conclusion: Our preliminary results show UPDRS was unable to track the worsening symptoms in two patients, which hindered their DBS programming. This illustrates that DBS programming will benefit from a more objective kinematic assessment tool that can track gait changes more specifically. The PKMAS carpet can give real-time feedback to the clinician to aid in more effective DBS programming.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS047388

Title: Data-driven statistical method for predicting the outcomes of combined pharmacologic and deep brain stimulation therapy for Parkinson’s disease

Authors: R. R. SHAMIR[1], T. DOLBER[1], A. M. NOECKER[1], A. M. FRANKEMOLLE[1], B. L. WALTER[1], *C. C. MCINTYRE[2]
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Abstract: Deep brain stimulation (DBS) of the subthalamic region is an effective treatment for the motor symptoms of advanced Parkinson's disease (PD). Following the surgery, the neurologist is faced with the challenge of balancing both the patient’s pharmacologic and stimulation treatments to maximize therapeutic benefit and minimize side effects. This complex process is currently driven by clinical experience and intuition, typically incorporating guidelines based on previous empirical studies. Unfortunately, this treatment optimization process often requires time-consuming follow-up visits with the patient because of the extremely large treatment parameter space. In response to these limitations, clinical decision support systems (CDSS) that incorporate patient-specific computer models to help customize DBS parameter

settings to the patient have been developed, and the first generation of commercial DBS CDSS are now available in Europe. However, these current systems only provide guidance regarding electrical stimulation, ignoring the pharmacology side of patient management. Therefore, we set out to define the foundation for a comprehensive PD CDSS that couples both stimulation and medication variables. We developed a linear function that relates the DBS parameters, the levodopa dosage, and patient-specific preoperative clinical data with the actual treatment motor outcomes. Our novel CDSS also incorporates image-based patient-specific computer models of DBS activation volumes in a multi-linear regression analysis. The resulting predictor function was highly correlated with the actual motor outcomes ($r = 0.76$; $p<0.05$). These results demonstrate that the outcomes of a combined pharmacologic-DBS therapy can be predicted with a PD CDSS. Such data-driven statistical systems have potential to facilitate patient-specific treatment optimization.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.10/O4

Topic: C.03. Parkinson’s Disease

Support: Michael j. Fox foundation

The Bachmann-Strauss Dystonia & Parkinson's Foundation

Title: Long term-cortical and subcortical recordings in Parkinson’s disease patients using a totally implanted device

Authors: *C. DE HEMPTINNE[1], N. SWANN*[1], J. OSTREM[2], M. SAN LUCIANO[2], N. GALIFIANAKIS[2], P. STARR[1]
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Abstract: * contributed equally Objectives: To assess the feasibility of chronic cortical and subcortical recording in humans with Parkinson’s disease (PD) using a novel bidirectional neural

interface, and to study chronic effects of deep brain stimulation (DBS) and antiparkinsonian medication on the motor network. Background: There is growing evidence for increased neuronal synchronization throughout the basal ganglia-thalamocortical loop in PD patients, especially in the beta band (13-30 Hz), that is reduced by medication and DBS. Most of this evidence has come from intraoperative studies or early postoperative recordings. Both these techniques are complicated by logistical constraints and cannot be dissociated from the acute brain physiology changes caused by edema around the recently inserted DBS electrodes. To overcome these limitations, we investigate the effect of antiparkinsonian therapies on cortical and subcortical signals using a novel fully implantable device, which has the capability of recording and storing data in addition to delivering therapeutic DBS. Methods: Three PD patients with severe motor fluctuations and minimal tremor have been each implanted with three electrodes; two DBS electrodes implanted bilaterally in the subthalamic nucleus (STN) and one subdural quadripolar strip electrode placed unilaterally over the primary motor cortex (M1). The cortical and ipsilateral STN electrodes were attached to an investigational, totally implanted neural interface capable of both recording and stimulation. Cortical and subcortical LFPs were recorded simultaneously in the outpatient clinic over multiple visits, on and off medication and on and off therapeutic stimulation. Signals were sampled at 800 Hz, stored internally and downloaded noninvasively by radiotelemetry. Results: Both cortical and subcortical signals were stable over time. In the STN, levodopa reduced low beta power (13-20 Hz), as previously described in acute recordings, but also reduced low gamma power (40 -70 Hz). In contrast levodopa did not modulate M1 beta power. Similarly, therapeutic DBS reduced beta and low gamma power in STN but did not affect M1 power. M1-STN coherence in the beta band was also reduced by levodopa. In one patient with prominent dyskinesias, a narrowband gamma peak (70 Hz) was consistently observed in M1 in the on but not in the off medication state. Discussion: These are the first chronic cortical and subcortical recordings in PD from a totally implanted device. They suggest that antiparkinsonian therapies have different effects on STN and cortical activities, decreasing beta oscillations in STN only, and decoupling STN-M1 interactions in the beta band.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson's Disease

Support: NIH RO1 NS 069779

Title: Electrocorticography reveals cortical characteristics of resting tremor in Parkinson's disease

Authors: *S. E. QASIM, C. DE HEMPTINNE, N. C. SWANN, P. A. STARR
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Abstract: Introduction: Resting tremor of 4-8 Hz is one of the cardinal motor signs of Parkinson's disease (PD). Tremor is mysterious in that it fluctuates spontaneously, does not increase in severity with increased dopamine denervation (though some denervation is necessary for tremor to occur), and never occurs in 1/3 of PD patients. These observations have led to the hypothesis that tremor may act as a compensatory mechanism to reduce the excessive motor network synchronization that characterizes PD. Methods: We investigated the spontaneous onset of tremor by recording electrocorticography (ECoG) using a 6 contact subdural electrode strip temporarily placed over the precentral and postcentral gyrus of patients undergoing deep brain stimulation (DBS) surgery. Recordings were made while patients were awake and at rest with eyes open, off of antiparkinsonian medications. Tremor was identified using a combination of electromyography measurements from the bicep, wrist extensor, and wrist flexor muscles and wrist accelerometery. ECoG recordings were compared during nontremor versus tremor epochs using measures of power spectral density (PSD) and phase-amplitude coupling (PAC). Results: We analyzed 19 intraoperative recordings in 9 patients with PD that contained intermittent epochs of tremor. Within patient comparisons revealed significant difference only in the contact over primary motor cortex (M1). We found that average alpha (8-12 Hz) and low beta (13-31 Hz) log power decreased significantly during tremor. 8 out of 9 patients showed none to very little PAC at rest. In those patients we found that the coupling of phase in high beta (27-32 Hz) to the amplitude of high gamma (50-200 Hz) increased during epochs of tremor. One patient who did have significant coupling at rest, however, showed large decreases in beta phase to gamma amplitude coupling. Conclusions: We used ECoG in patients undergoing DBS surgery for PD, to measure changes in cortical activity between rest and tremor. Resting tremor modulated power in the alpha and beta bands of M1. It also increased the synchronization of high gamma amplitude to high beta phase, though this result was reversed in a patient who showed large PAC at rest.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson's Disease

Support: Medical Research Council

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Title: Parkinsonian and essential tremor pathophysiology

Authors: *H. CAGNAN[1], S. LITTLE[1], T. FOLTYNIE[2], P. LIMOUSIN[2], L. ZRINZO[2], M. HARIZ[2], B. CHEERAN[1], J. FITZGERALD[1], A. GREEN[1], T. AZIZ[1], P. BROWN[1]
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Abstract: Tremor is central to a wide range of pathologies. However, the mechanisms underlying tremor generation remain largely unknown. In this study, we address two fundamental issues 1- the role of different deep brain stimulation targets in tremor generation, and 2- pathophysiological differences between Parkinsonian and essential tremor circuits. We hypothesised that stimulation delivered at certain parts of a tremor cycle should interact with oscillatory neural activity driving tremor, which would then be reflected as an instantaneous change in the temporal profile of tremor. Accordingly, we stimulated fifteen patients with Parkinson's disease with either thalamic or subthalamic electrodes (13 male and 2 female patients, age: 50-77 years) and ten patients with essential tremor with thalamic electrodes (9 male and 1 female patients, age: 34-74 years). We show that both thalamic and subthalamic stimulation significantly entrains tremor in patients with Parkinson's disease. Critically, though, there was no significant stimulation timing dependent change in instantaneous tremor amplitude when compared to tremor amplitude variability observed without stimulation. This is in stark contrast to essential tremor where thalamic deep brain stimulation at tremor frequency both entrained tremor and modulated instantaneous tremor amplitude depending on the tremor phase at which stimulation was applied. Parkinsonian and essential tremor also differed in the relative tolerance of their amplitudes (i.e. tremor severity) to spontaneous changes in instantaneous tremor frequency, with the amplitude of parkinsonian tremor demonstrating greater tolerance to spontaneous changes in instantaneous tremor frequency than in essential tremor. Based on these results we conclude that parkinsonian tremor is driven by a neural network, which includes the subthalamic nucleus and ventrolateral thalamus and has broad frequency-amplitude tolerance. We propose that it is this tolerance to changes in tremor frequency that dictates that Parkinsonian tremor may be significantly entrained by low frequency stimulation without amplitude modulation. In contrast, the neural circuits influenced by low frequency thalamic stimulation in

essential tremor have a narrower frequency-amplitude tolerance so that tremor entrainment through extrinsic driving is necessarily accompanied by amplitude modulation. Such differences in Parkinsonian and essential tremor will be important in selecting future strategies for closed loop deep brain stimulation for tremor control.

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Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson's Disease

Title: Impedance reliability during neurostimulator replacement: Activa to activa vs. soletras to activa

Authors: ***E. L. HARGREAVES**[1], E. M. FEINSTEIN[2], R. J. DITOTA[1], S. WONG[2], S. F. DANISH[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 (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. Here, we compare the difference between impedance assessments of Soletras to Activa SCs versus Activa PC/SCs to Activa PC/SCs during surgical generator replacement procedures. If the Activa family of devices is more accurate then there should be less of a difference and greater similarity moving from an Activa device to an Activa device then moving from a Soletra to an Activa SC. The Soletras were replaced in pairs (24 readable Soletras from 13 implanted individuals), and as such, a number of the devices were considered functioning at a normal battery charge (8/24), with only their contralateral counterparts failing. This is in contrast to the Activa generator replacements, where all 10 devices (9/10 dual channel PCs and 1/10 SC from 10 implanted individuals) were at

or near the “Elective Replacement Indicator” (ERI) thresholds. Analyses were performed on the monopolar impedances recorded immediately prior to deactivation and upon the intraoperative test of the new devices. Average time between Soletra to Activa SC assessments was 102min, which was not different from that between Activa to Activa assessments at 76min. Although the difference between monopolar impedance assessments for Soletras to Activa SCs (mean 63 Ohms) was similar to that of the Activa to Activa devices (mean 65 Ohms) the variability was statistically greater as analyzed by the absolute difference (mean 101 sem 12.4 Ohms compared to mean 76 sem 6.4 Ohms respectively). This was further supported by stronger correlations between impedance values before and during replacement procedures of the Activa to Activa devices (r=.93) over the Soletra to Activa SC devices (r=.73). Thus, these clinical results are consistent with our previous bench tests indicating that the Activa devices were more accurate then their predecessors, even when close to ERI thresholds. Differences in Soletra impedance as battery life declines may translate to degradations in clinical efficacy during this time and may necessitate compensatory programming changes, which may further need to be tempered upon replacement.

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Poster

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Greater St. Louis Chapter of the APDA

McDonnell Center for Higher Brain Function

Title: Distinct patterns of phase-amplitude coupling within the subthalamic nucleus in Parkinson disease

Authors: S. RYU[1], R. MURPHY[3], M. USHE[4], J. L. DOWLING[3], K. M. RICH[3], J. S. PERLMUTTER[5], S. CHING[2], *S. A. NORRIS[4]
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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is a common and effective treatment for Parkinson disease (PD). Local field potentials (LFP) recorded from the STN in PD patients have revealed elevated power in the beta (13-30 Hz) band (for review, see Weinberger et al, 2009). Recent work suggests that clinical outcomes correlate with placement of a DBS electrode in relation to the region of highest beta oscillation in the dorsolateral STN (Zaidel et al, 2010). Here, we show the presence of distinct patterns of LFP phase-amplitude coupling within the STN. We obtained microelectrode recordings in 13 PD patients undergoing bilateral implantation of DBS electrodes in STN. For each patient, microelectrode recordings were obtained at different depths along a single trajectory aimed through dorsolateral STN (n = 26 electrode trajectories). All patients were awake and resting on the operating table with eyes open throughout recording. Data were analyzed using standard Fourier-based methods and phase-amplitude coupling techniques (Tort et al, 2010). Two distinct, significant ($P<0.01$) phase-amplitude coupling patterns were observed within the STN of the majority of patients (22/26 trajectories):(i) gamma frequency (>30 Hz) phase coupled to high frequency oscillation (HFO, > 200 Hz); and (ii) beta frequency (13-20Hz) phase coupled separately to activity centered at 150 Hz and 200 Hz, but spectrally distinct from HFO. We hypothesize that the HFO activity reflects action potentials manifesting in the LFP. Thus, the observed activity implies the rhythmic firing of neurons in multiple spectral bands in the STN, depending on spatial location. The differential spatiotemporal activity within the STN implicates sub-STN circuit mechanisms in the pathophysiology of PD. Such new spectral markers may help guide DBS electrode placement.

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Poster

412. Parkinson's Disease: Clinical Therapies

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GAANN Fellowship

Title: Comparison of deep brain stimulation neural activation models developed with 7T MRI data

Authors: *K. GUNALAN[1], A. CHATURVEDI[1], Y. DUCHIN[2], G. SAPIRO[3], N. HAREL[2], C. C. MCINTYRE[1]
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Abstract: Deep brain stimulation (DBS) has been shown to modulate axonal activity within the vicinity of the active electrode contact(s). Computational modeling provides for the ability to estimate the extent of axonal activation in response to extracellular stimulation, as first described by McNeal in 1976. In this study we compare two previously described methods of estimating axonal activation in the context of DBS: the McNeal-based approach and the activation volume predictor function. The McNeal-based approach couples DBS electric field modeling with multi-compartment cable axon models and thus provides the most detailed method of estimation. Activation volumes are based on classifiers that can approximate the spatial extent of axonal activation as a function of the stimulation parameter settings, which is computationally less intensive than the McNeal-based approach. We compared these models in a Parkinson's disease DBS patient using high-resolution (7T) magnetic resonance imaging data. We used probabilistic tractography to reconstruct the hyperdirect and subthalamopallidal pathways, which are believed to mediate the therapeutic benefit of subthalamic DBS, and the internal capsule, activation of which causes side effects. We modeled current controlled stimulation and, using the two methods, calculated the pathway activation patterns for clinically relevant stimulation parameter settings. The activation volume-based calculations were found to overestimate results compared to the McNeal-based approach. Despite this shortcoming, activation volumes provide for real-time visualization of the effects of stimulation parameters changes on the pathway activation patterns.

Disclosures: **K. Gunalan:** None. **A. Chaturvedi:** None. **Y. Duchin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Surgical Information Sciences, Inc. **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.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

412. Parkinson's Disease: Clinical Therapies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 412.16/O10

Topic: C.03. Parkinson’s Disease

Support: John A. Blume Foundation

Medtronic, Inc. provided the investigational devices only

Title: Effects of low (60 Hz) and high (130/140 Hz) frequency STN DBS on upper extremity movement velocity and STN beta band power in Parkinson’s disease

Authors: ***Z. BLUMENFELD**[1], A. VELISAR[1], M. MILLER KOOP[1], L. SHREVE[1], E. QUINN[1], B. HILL[1], C. KILBANE[1], C. RODRIGUEZ[1], J. HENDERSON[2], H. BRONTE-STEWART[1]
[1]Neurol., [2]Neurosurg., Stanford Univ., Stanford, CA

Abstract: Objective: High frequency subthalamic nucleus deep brain stimulation (HF STN DBS) attenuates resting STN local field potential (LFP) power in the 13 - 30 Hz (beta) band and improves the cardinal motor signs of Parkinson’s disease (PD). Low frequency (60 Hz) STN DBS has not been useful clinically except for aspects of speech and gait. We have shown that 60 Hz DBS amplifies resting LFP peak power (Bronte-Stewart et al., SfN Abstract 2013). Therefore, we hypothesize that HF DBS, but not 60 Hz DBS, would improve limb bradykinesia. We report preliminary data on the effect of 60 Hz versus 140 Hz DBS on STN beta band power during movement. Methods: Five PD subjects (nine sides), off-medication at least one month

after DBS implantation surgery, performed a seated repetitive wrist flexion-extension (rWFE) task without DBS (baseline), and during randomized presentations of 60 Hz DBS, or HF (130 or 140 Hz) DBS (each either 2 or 3V). In three cases, STN LFPs were also recorded from electrodes 0 - 2 of the DBS lead (model 3389, Medtronic, Inc.) and from the investigational Activa® PC+S system via telemetry (FDA, IDE, IRB, and CA Medicare approved) while DBS was applied through electrode 1. Hand angular velocity was recorded using solid-state gyroscopic sensors (Motus Bioengineering, Inc.) and was acquired using Spike software (Cambridge Electronic Design, Inc.). Root-mean-square angular velocity (Vrms) was calculated in MATLAB (The MathWorks, Inc.). Results: Both HF DBS and 60 Hz DBS significantly increased Vrms compared to baseline, $p < 0.05$. There was no significant difference in the improvement during 60 Hz versus HF DBS. Preliminary results of movement-related STN LFPs recordings revealed no attenuation of baseline-movement-related beta band power during 60 Hz DBS and concomitant improvement in Vrms, whereas HF DBS attenuated baseline beta band power and improved Vrms (2/3 sides). For the third side, HF DBS improved Vrms to a greater extent than 60 Hz DBS, and there was attenuation of baseline-movement-related beta band power during both HF and 60 Hz DBS. Conclusions: Contrary to our hypothesis, both 60 Hz and HF DBS improved upper extremity bradykinesia to the same extent. Preliminary data suggests that improvement in Vrms during 60 Hz DBS may not be associated with attenuation of beta band power as it was during HF DBS. These results suggest that the attenuation of STN beta band power may not be causal to the improvement in bradykinesia and that 60 Hz DBS may be useful even for appendicular motor signs in PD.

Disclosures: Z. Blumenfeld: None. **A. Velisar:** None. **L. Shreve:** None. **M. Miller Koop:** None. **E. Quinn:** None. **B. Hill:** None. **C. Kilbane:** None. **C. Rodriguez:** None. **J. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Nevro Corp.. **H. Bronte-Stewart:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Topic: C.03. Parkinson’s Disease

Support: John A. Blume Foundation

Medtronic INC provided Activa PC+S Neurostimulator's for this study, but no financial support

Title: STN LFP recordings from the implanted Activa® PC+S neurostimulator system in freely moving PD subjects reveal conserved resting state profiles while lying, sitting or standing and voltage dependent beta band attenuation during 140 Hz DBS

Authors: *E. J. QUINN[1], Z. BLUMENFELD[1], L. SHREVE[1], A. VELISAR[1], M. KOOP[1], C. KILBANE[1], J. HENDERSON[2], C. RODRIGUEZ[1], B. HILL[1], H. BRONTE-STEWART[1]
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Abstract: Objective: To determine whether the resting state subthalamic nucleus (STN) alpha/beta band is similar lying, sitting or standing in PD subjects (PDs) with the implanted investigational Activa® PC+S neurostimulator system (Medtronic Inc.). Additionally, we investigated whether signal to noise characteristics of the implanted neurostimulator system with local field potential (LFP) sensing capability allowed discernable recordings during high frequency (HF) deep brain stimulation (DBS) at different voltages. Methods: STN LFPs were recorded from electrodes 0-2 from the DBS lead (model 3389, Medtronic, Inc.) using the Activa® PC+S system via telemetry (FDA IDE, IRB and CA Medicare approved) from five PDs (10 STNs) during lying, sitting, and standing (no DBS) and before, during and after randomized periods of 140 Hz (60 microsec) DBS at 1V and 3V through electrode 1 (while seated). PDs were off medication (>24/12 hours for long-acting/short-acting). Movement was monitored using angular velocity sensors, EMG, and continuous video, synchronized with LFPs. Data was carefully parsed to avoid any movement or electrical artifact during resting states. Alpha/beta (8-35 Hz) and beta band (13-30 Hz) power was calculated using previously published methods. Results: There was no difference in resting alpha/beta power during standing lying and sitting postures (P=0.967, 8-35 Hz) for the 10 sides. There was significant attenuation of beta band power during HF DBS at 3V but not at 1V (P=0.0002 at 3V, P= 0.116 at 1V, 13-30 Hz) in 6/10 sides where recording quality was acceptable. Electrical artifact limited the ability to discern neural signals during DBS in two sides and was worse at higher voltages. One subject was removed from unilateral stimulation analysis due to unconfirmed lead labelling. Conclusions: This is the first report to demonstrate concurrent and synchronized STN neural recordings and kinematic data in freely moving PDs from the implanted Activa® PC+S neurostimulator system in different resting postures and during HF DBS. We show that the LFP spectral profile is conserved during lying, sitting, and standing postures, and appears to be a robust property of the resting state in PD. We confirm our previous finding that beta band power is attenuated during HF DBS in a voltage dependent manner. Additionally, we will present details of the limitations encountered in our early experience with the Activa® PC+S neurostimulator system.

Disclosures: E.J. Quinn: None. **Z. Blumenfeld:** None. **L. Shreve:** None. **A. Velisar:** None. **M. Koop:** None. **C. Kilbane:** None. **J. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

Nevro Corp, Intelect Medical. F. Consulting Fees (e.g., advisory boards); Nevro Corp, Intelect Medical. **C. Rodriguez:** None. **B. Hill:** None. **H. Bronte-Stewart:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 412.18/O12

Topic: C.03. Parkinson's Disease

Support: UROSARIO GRUPO NEUROS

Title: Development of devices for movement assessment in clinical settings for Parkinson's disease

Authors: *J. A. RAMIREZ[1], M. R. TORRES-NARVÁEZ[5], N. FLOREZ[2], N. MORALES[3], D. QUIROGA[4], G. LUNA-CORRALES[5]
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Abstract: We have developed an affordable device for the measurement of movement of easy access that can be used in clinical practice in the diagnosis, follow-up, and evaluation of Parkinson's patients. We are using this device in a pilot study with Parkinson's patients in the Hospital of Barrios Unidos in Bogota Colombia, which is part of the health network of hospitals of UROSARIO. Experimental Design. Our device consists of the accelerometer/gyroscope chip MPU-6050 mounted on a card with a serial I2C reading protocol. An ARDUINO UNO microcontroller with an ATMEL 380 chip for data acquisition and control and a Xbee Pro Series 2B for inalambric transmission. We use open source processing and python programs for signal conditioning and processing. Pilot Study. In all the patients with Parkinson's disease as well as the controls evaluated in this study we apply the miniBESTest (Balance Evaluation System Test) developed by Oregon Health Science University (www.bestest.us) to evaluate balance taking into account biomechanics, stability limits, postural responses, anticipatory postural adjustments, sensorial orientation and dynamic balance during walking. Balance and postural protocols. We use sit-stand-sit transitions, standing on a force-platform with eyes open and closed. We use an elastic belt located between L3-L5 where our device is placed. We also use a gripping protocol to assess isometric grip force, load force, acceleration in three dimensions, and grip tremor amplitude . In this case, we place the device in the wrists of the subjects. For the sit-stand-sit

transitions we are measuring time to peak, antero-posterior acceleration, amplitude, tilt and jerk. For the standing position we correlate the force measurement with mean velocity, F95% and jerk as an indication of the smoothness of postural changes. With these techniques we wish to contribute to the establishment of protocols of evaluation of kinetic and kinematic variables of movement in Parkinson's disease using affordable devices in processes of rehabilitation. .

Disclosures: J.A. Ramirez: None. **M.R. Torres-Narváez:** None. **N. Florez:** None. **N. Morales:** None. **D. Quiroga:** None. **G. Luna-Corrales:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.19/P1

Topic: C.03. Parkinson's Disease

Support: University of Kentucky Startup funds (CVH)

National Center for Advancing Translational Sciences, through grant UL1TR000117

Title: Employing deep brain stimulation surgery as a platform for implanting peripheral nerve grafts into the central nervous system

Authors: C. G. VAN HORNE[1,2], J. T. SLEVIN[3], J. E. QUINTERO[1], *G. A. GERHARDT[1], J. A. GURWELL[3]
[1]Anat, Neurobiol & Neurol, Univ. Kentucky Med. Ctr., LEXINGTON, KY; [2]Neurosurg., [3]Neurol., Univ. of Kentucky, Lexington, KY

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. We have an ongoing clinical trial with the primary endpoint 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. Schwann cells from the peripheral nervous system may serve as potential sources of neurotrophic factors including GDNF, NGF, BDNF, and NT-3, and peripheral nerve grafts to the CNS may provide an opportunity to directly deliver neurotrophic factors in areas affected by neurodegenerative diseases. Multi-stage, DBS surgery targeting the subthalamic nucleus was performed using standard procedures. After the DBS leads were implanted, a section of sural nerve (approximately 5mm in length) containing Schwann

cells was excised and unilaterally delivered, using a custom-designed cannula, into the area of the substantia nigra. Adverse events were continuously monitored. Our secondary aim was to measure changes in motor function through assessment of on a Unified Parkinson's Disease Rating Scale (UPDRS) evaluation before surgery and at 1, 3, 6, 9, and 12 months after surgery. We have successfully completed peripheral-nerve-graft surgery in 6 of 6 participants. Immediate, post-operative magnetic resonance scans have not indicated evidence of abnormal tissue disruption. Participants who completed nine months in the study reported comparable adverse effects to standard DBS surgery. In addition, UPDRS Part III scores off medication/off stimulation decreased after nine months (36 ± 8 baseline vs. 25 ± 13, mean ± SD; N=4). Meanwhile, scores on medication and on stimulation improved by 8 points (16 ± 11 baseline vs. 8 ± 6, nine months), and daily levodopa equivalents decreased from a mean of 844 ± 691 mg at baseline to zero after nine months. We have begun proteomic studies to examine expressions of neurotrophic-factor proteins in the peripheral nerve grafts. During the initial months of the study, we have observed a limited number of adverse events along with some improvements on UPDRS evaluations but on-going assessments will help gauge the safety and feasibility of implanting peripheral nerve tissue in conjunction with DBS surgery and the potential benefits these grafts may provide.

Disclosures: **C.G. van Horne:** Other; Educational support from Medtronic. **G.A. Gerhardt:** 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.; Medtronic and Eli Lilly. **J.T. Slevin:** None. **J.E. Quintero:** None. **J.A. Gurwell:** None.

Poster

412. Parkinson's Disease: Clinical Therapies

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Program#/Poster#: 412.20/P2

Topic: C.03. Parkinson's Disease

Support: National Center for Advancing Translational Sciences, through grant UL1TR000117

University of Kentucky start-up funds (CVH)

Title: Microelectrode recordings of the globus pallidus under sevoflurane anesthesia in Parkinson's disease patients

Authors: *J. E. QUINTERO[1], G. A. GERHARDT[1,2], C. G. VAN HORNE[1,2]
[1]Anat. & Neurobio., Univ. Kentucky, Lexington, KY; [2]Neurosurg., Univ. of Kentucky, Lexington, KY

Abstract: Recent reports have provided justification for using the internal globus pallidus (GPi) over the subthalamic nucleus (STN) as a target for implanting DBS leads. A consistent challenge for Parkinson's disease patients in DBS surgery is the awake portion of the surgery. An alternative would be to implant DBS leads in patients who are asleep. One concern for these types of surgeries is the ability to physiologically delineate the structures of the GPi and external globus pallidus (GPe) before securing the DBS lead. Our goal was to assess and characterize the electrophysiology of the GPe/GPi with microelectrode recordings and micro and macro stimulation during implantation in patients undergoing DBS surgery while under general anesthesia. We performed a retrospective study and comparison of dystonic and Parkinson's disease patients who underwent bilateral pallidal DBS placement with MER. Single-pass MER performed with tungsten-tipped microelectrodes to map GPe and GPi. MER recordings were performed on subjects who were either awake or under general anesthesia. We employed electrical micro and macro stimulations to obtain adverse effect profiles. We observed neuronal firing patters in the GPe/GPi of patients who were awake and under general anesthesia. Activation of the internal capsule was monitored with micro and macro stimulation. The identified firing patterns corresponded with palladial structures and borders, and the MER and stimulation results were used intraoperatively in deciding DBS lead placements. MER recordings of GPe/GPi along with micro/macro stimulation in asleep patients can help facilitate intraoperative DBS lead placement. Future studies will have to determine if palladial DBS lead placement, in patients under general anesthesia, can provide an effective means of avoiding the awake experience of DBS lead placement.

Disclosures: J.E. Quintero: None. **G.A. Gerhardt:** 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.; Medtronic and Eli Lilly &Co. **C.G. van Horne:** Other; Medtronic-Educational Support.

Poster

412. Parkinson's Disease: Clinical Therapies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 412.21/P3

Topic: C.03. Parkinson's Disease

Title: Development of a sheep platform and behavioral monitoring methods for assessing deep brain stimulation therapies and devices for movement disorders

Authors: *R. S. RAIKE[1], Y. ZHAO[2], L. LENTZ[1], W. SCHINDELDECKER[1], M. KELLY[1], D. E. NELSON[1]
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Abstract: Preclinical animal models are essential for successful development of safe and effective commercialized CNS therapies. Objective data from animal models establish proof of concept evidence and provide critical inputs to the design of later phase clinical studies. In medical device development, a large animal with an intact nervous system is preferred because it permits use of human-scaled devices and controlled testing that cannot be replicated with computer modeling or bench-testing. Therefore, we established an in-house sheep platform for early-phase testing of deep brain stimulation (DBS) therapy concepts and devices within the movement disorders space. In twelve animals commercial DBS leads with four active contacts were targeted to the subthalamic nucleus (STN), a common stimulation target in Parkinson disease. Overall, DBS lead implantation was not associated with remarkable neurological or histopathological complications. Assessments of targeting using standard comparisons of pre and post-operative brain images indicated that accuracy was comparable to clinical experience. Methods were developed to quantitatively assess motor behavior of chronically-implanted animals in the awake state. In open and blinded settings, we consistently found that motor behavior responses to STN stimulation significantly depended on the stimulation contact selected and parameters tested, including voltage, pulse width and frequency. Quantitative electromyographic assessments confirmed the motor behavior findings. This work establishes in-house capabilities for controlled testing of emerging DBS therapy concepts and device prototypes. Further work is ongoing to test prototype devices and develop additional objective physiological monitoring methods and biomarkers.

Disclosures: **R.S. Raike:** A. Employment/Salary (full or part-time):; Medtronic Neuromodulation. 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.; Medtronic Neuromodulation. **Y. Zhao:** A. Employment/Salary (full or part-time):; Medtronic, Neuromodulation. **L. Lentz:** A. Employment/Salary (full or part-time):; Medtronic, Neuromodulation. **W. Schindeldecker:** A. Employment/Salary (full or part-time):; Medtronic, Neuromodulation. **M. Kelly:** A. Employment/Salary (full or part-time):; Medtronic, Neuromodulation. **D.E. Nelson:** A. Employment/Salary (full or part-time):; Medtronic, Neuromodulation.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

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Program#/Poster#: 413.01/P4

Topic: C.03. Parkinson's Disease

Support: Young Faculty Program Rita Levi-Montalcini (Italian Ministry of Education)

Bio@SNS Scuola Normale Superiore

Title: Modelling α-synuclein aggregation in a cell system

Authors: *E. COLLA, V. LIVERANI, A. CATTANEO
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Abstract: A better understanding of the cellular mechanisms involving the formation of α-synuclein (αS) aggregates, a pathological hallmark of α-synucleinopathies, is necessary to elucidate the underlying pathogenesis and develop effective therapeutic strategies. While animal models partially recapitulate the formation of αS fibrils in neurons as seen in humans, it has been very difficult to obtain reliable and reproducible data using cell models. Cell models would be more amenable for detailed mechanistic studies. Because of recent evidence that linked association of αS aggregates with the endoplasmic reticulum (ER)/microsomes vesicles to neurodegeneration in Prp A53T αS transgenic mice, we investigated if targeted expression of αS in the ER could lead to accumulation of ER-associated αS oligomers/aggregates in a cell culture system. Human wild-type or mutated (A53T or A30P) αS was cloned in the ER expression vector pCMV *myc*/ER in frame with an ER localization and retention signal. A Myc tag at the C-terminal of αS was also included. Transfection of SH-SY5Y neuroblastoma cell line with ER-targeted forms of αS led to expression of αS in the ER as shown by confocal colocalization with the ER marker grp94. While most of the transfected cells showed a dot-like staining typical of αS, a small percentage of cells also accumulated compact structures with a round shape, resembling aggregated species of αS and positive for antibodies against αS and Myc. αS staining was particularly intense at the periphery of the ring with a more opaque core at the center. These species colocalized with the ER and were seen in cell bodies as well as in processes. No major differences were seen by immunofluorescence, between the expression of wild-type and mutated forms of αS. At the same time, transfection with wild-type or mutated αS lacking the ER retention signal that leads to secretion of the protein failed to produce any αS staining or inclusions. Because of transient transfection did not provide a homogenous population of cells expressing αS in the ER and consequently forming ER-associated αS aggregates, we are now

developing inducible and stable cell lines expressing human wild-type, A53T and A30P mutated αS in the ER. We expect to be able to obtain several lines for each αS isoform with a different gradient of accumulation of αS aggregates. We plan to use this model to study cellular pathways affected by αS aggregation and to identify strategies to modify fibrils accumulation.

Disclosures: E. Colla: None. **V. Liverani:** None. **A. Cattaneo:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.02/P5

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS073740

Title: Extracellular ATP induces intracellular alpha-synuclein accumulation via P2X1 receptor-mediated lysosomal dysfunction

Authors: *M. GAN[1], S. MOUSSAUD[1], P. JIANG[1], P. J. MCLEAN[1,2]
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Abstract: The pathological hallmark of Parkinson's disease (PD) is the accumulation of alpha-synuclein (αsyn) in susceptible neurons in the form of Lewy bodies and Lewy neurites. However, the etiology of PD remains unclear. Because brain injury has been suggested to facilitate αsyn aggregation, we investigated whether cellular breakdown products from damaged cells can act on neighboring healthy cells and cause intracellular αsyn accumulation/aggregation. Using two neuronal cell models we found that extracellular ATP-induced a 2-fold increase in intracellular αsyn levels between 24 to 48 hours after treatment. Further investigation revealed that the observed αsyn accumulation is a result of lysosome dysfunction caused by extracellular ATP-induced elevation of lysosomal pH. Interestingly, P2X1 receptor, a subtype of ATP-selective receptors that function as Ca^{2+} permeable, ligand-gated ion channels appear to mediate the cells' response to extracellular ATP. However, although Ca^{2+} influx via P2X1 receptors is necessary for αsyn accumulation, Ca^{2+} influx per se is not sufficient for increased αsyn accumulation. These findings provide new insight into our knowledge of the role of P2X receptors in PD pathogenesis and may be helpful in identifying new therapeutic targets for PD.

Disclosures: **M. Gan:** None. **S. Moussaud:** None. **P. Jiang:** None. **P.J. McLean:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.03/P6

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, R01ES014826

Title: The saturated free fatty acid palmitate increases alpha-synuclein expression levels-relevance to synucleinopathies

Authors: ***O. GHRIBI**[1], J. SCHOMMER[2], S. RAZA[2]
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Abstract: Accumulation of the A-synuclein protein in Lewy body inclusions is a hallmark of a group of disorders collectively known as the synucleinopathies. These disorders include idiopathic Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. Lewy bodies-containing a-synuclein are also frequently found in Lewy body variants of Alzheimer's disease. Despite extensive research, no disease-modifying therapy is currently available for synucleinopathies and the search for diagnostic tests and biomarkers are still under development. The search for disease-modifying therapies or diagnostic markers would benefit from identification of factors that promote over-production of A-synuclein protein or from elucidation of cellular mechanisms that regulate the transcription of A-synuclein. Identification of such factors and underlying cellular mechanisms may help in understanding the pathogenesis of synucleinopathies and designing therapeutic agents that can prevent, reverse, or stop the over-production of a-synuclein and ultimately protect against synucleinopathies. In this study, we determined the effect of a diet rich in the saturated free fatty acid (sFFA) palmitate on a-synuclein *in vitro* and *in vivo*. We found that incubation of human neuroblastoma SH-SY5Y cells with palmitate increased expression levels of a-synuclein. We also fed wild type mice with a diet rich in palmitate for 3 months and found that this diet increases the expression levels of a-synuclein in hippocampus, cortex and substantia nigra. The etiology of synucleinopathies is not well defined but it is likely that the interplay between environmental (chemical and dietary) factors and genetic susceptibilities plays a key role in the pathogenesis of this disease. Our data

suggest that intake of diets with a high content of palmitate increase A-synuclein production and may promote synucleinopathies.

Disclosures: O. Ghribi: None. **J. Schommer:** None. **S. Raza:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.04/P7

Topic: C.03. Parkinson's Disease

Support: NIH/NIA 5R01 AG13966

Title: The role of dopamine in generating toxic oligomeric conformers of alpha-synuclein

Authors: *D. E. MOR[1], E. TSIKA[2], J. R. MAZZULLI[3], J. H. WOLFE[1], H. ISCHIROPOULOS[1]
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Abstract: Parkinson's disease (PD) is defined by marked loss of dopamine (DA) producing neurons in the substantia nigra (SN) and an abundance of Lewy body inclusions comprised of aggregated α-synuclein (α-syn) protein. The role of α-syn in neurodegeneration remains a major research area. Growing evidence suggests oligomeric intermediates that form during aggregation of α-syn are neurotoxic. Kinetic stabilization of α-syn oligomers through a non-covalent interaction with oxidized DA may contribute to neurotoxicity. However, it remains unknown if DA interacts with α-syn oligomers *in vivo*, and if DA-stabilized species induce cellular injury. To investigate the role of DA in promoting α-syn toxicity, we are using an established mouse model of α-synucleinopathy expressing human α-syn with the A53T familial PD mutation. In this model, α-syn oligomers are formed in the SN without associated cell loss. Using a novel lentiviral approach to increase DA production in the SN of A53T mice, we are testing the effects on α-syn oligomers and cell viability. The virus carries the gene for tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, with mutations at residues R37E, R38E (TH-RREE) such that feedback inhibition by DA is greatly reduced. Preliminary data indicate that TH-RREE significantly increases TH expression and striatal catecholamine levels compared with an empty vector control. Intriguingly, there is also an increase in steady-state levels of α-syn oligomers, and a shift in oligomer size towards larger Stokes radius. Formation of large intracellular aggregates of α-syn was not observed, consistent with DA-mediated stabilization of oligomers.

Moreover, the number of SN neurons is significantly reduced. Together, these data implicate DA and α-syn in a pathogenic mechanism that may underlie the selective loss of DA cells in PD.

Disclosures: D.E. Mor: None. **E. Tsika:** None. **J.R. Mazzulli:** None. **J.H. Wolfe:** None. **H. Ischiropoulos:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Support: FCT grant SFRH/BD/74881/2010

Title: The interplay between atp13a2 and alpha-synuclein: mitochondria dysfunction via endoplasmic reticulum stress

Authors: *T. LOPES DA FONSECA, T. OUTEIRO
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Abstract: A common pathological hallmark among several neurodegenerative diseases, including Parkinson's disease (PD), is the aberrant protein-protein interactions that result in disruption of several essential cellular functions. Misfolding and aggregation of α-synuclein (a-syn) has been extensively studied, as this is considered a central process in PD pathology. However, the interplay between a-syn and other PD-related proteins is still unclear. ATP13A2, a lysosomal transmembrane P5 ATPase, has been recently described to be part of the a-syn-interacting network, as it was able to rescue a-syn related toxicity in a yeast model. The physiological role of this protein is uncertain, but it is thought to play a role in metal homeostasis regulation, autophagy and mitochondrial function. One ATP13A2 PD-causative mutation consists in the duplication of residues 1632-1653 leading to a frameshift that shortens the protein size (ATP13A2 dup22). Here, we investigated the interplay between a-syn and ATP13A2, *in vitro*. A wide range of cellular effects was observed in human cells co-overexpressing a-syn and wild-type (WT) or ATP13A dup22. Briefly, cells displayed altered ER morphology, ER stress, and mitochondrial fragmentation. Interestingly, analysis of mitochondria bioenergetics showed that ATP13A2 dup22 reduces mitochondrial spare capacity and promotes the production of reactive oxygen species. Furthermore, in a cell-based a-syn aggregation paradigm, ATP13A2 dup22 significantly increased a-syn aggregation and promoted cytotoxicity. In total, our studies

identified a novel interplay between a-syn and ATP13A2, providing novel insights into the molecular basis of PD.

Disclosures: T. Lopes Da Fonseca: None. **T. Outeiro:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 413.06/P9

Topic: C.03. Parkinson's Disease

Title: Abnormal alpha-synuclein reduces nigral voltage-dependent anion channel 1 in sporadic and experimental Parkinson's disease

Authors: *Y. CHU, Y. HE, J. H. KORDOWER
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Abstract: Alpha-synuclein abnormality and mitochondrial dysfunction are considered as tow major contributors to Parkinson's disease. Their relationships are still unclear. The present study investigated alterations of voltage-dependent anion channel 1 (VDAC1) within remaining nigral dopaminergic neurons in patients with sporadic Parkinson's disease and rats with experimental Parkinson's disease as compared with controls. VDAC1 is a major component of the outer mitochondrial membrane known to regulate mitochondrial functions. Co-localization analyses revealed that VDAC1 immunoreactivity was severely reduced in the neuromelanin (NM)-laden neurons with alpha-synuclein inclusions in PD. Although there was cytoplasmic non-aggregative alpha-synuclein in age-matched controls, VDAC1 immunoreactivity in the alpha-synuclein positive nigral neurons was similar to the alpha-synuclein negative neurons. The VDAC1 existed in the fibers with fine granules alpha-synuclein but not in the fiber with accumulative and aggregative alpha-synuclein. Quantitative analyses revealed that the relative levels of VDAC1 were significantly decreased in PD nigral neurons when compared to age-matched controls. In PD, this decrease was significantly greater in nigral neurons with alpha-synuclein inclusions. In contrast, levels of MTC02, another general mitochondrial marker, was unchanged in neurons with alpha-synuclein aggregations that was similar to neurons without alpha-synuclein aggregations. Viral vector-mediated overexpression of mutant human alpha-synuclein (A30P) in rats resulted in significantly decreased VDAC1 in nigral neurons and striatal fibers. These results indicate that mitochondrial function associated with VDAC1 is decreased in sporadic and

experimental PD, and this decrease is associated with alpha-synuclein accumulation and aggregation.

Disclosures: **Y. Chu:** None. **Y. He:** None. **J.H. Kordower:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Greek General Secretariat for Research and Technology grant 2272 ARISTEIA ParkinsonTransMed

Greek General Secretariat for Research and Technology grant 09SYN-21-969 NoisePlus

Title: Transcriptional profiling of control and A53T-alpha synuclein human iPS cells differentiated towards midbrain dopamine neurons: A window to regulatory developmental pathways and A53T-associated dysfunction

Authors: ***R. MATSAS**[1], K. PRODROMIDOU[1], I. VLACHOS[2], A. HATZIGEORGIOU[2], G. KOUROUPI[1], E. TAOUFIK[1], K. TSIORAS[1]
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Abstract: Human embryonic stem cells (HUES) and induced pluripotent stem cells (hiPS) are characterized by their ability to self-renew and their potential to differentiate into specialized cell types. The ability to generate large numbers of early precursors and stage-specific differentiated cells renders them a valuable source for studies in developmental biology, applications in regenerative medicine or for modeling human diseases. In particular, hiPS provide an unprecedented opportunity to study otherwise inaccessible human neurons and their precursors while the generation of patient-specific hiPS can significantly contribute in our understanding of the molecular mechanisms underlying neurodegenerative diseases. We have generated midbrain dopamine neurons by directed differentiation of hiPS derived from fibroblasts of healthy individuals and Parkinsonian patients harbouring an autosomal dominant and highly penetrant A53T mutation in alpha-synuclein (A53T-αSyn). To identify novel differentiation-related critical genes and regulatory networks as well as to discover A53T-dysregulated pathways, we have

launched whole transcriptome analysis of hiPS-derived cells by new generation sequencing of coding and non-coding RNAs (long non-coding RNAs and miRNAs) at specific stages of differentiation (iPS, neural precursors and neurons). We have complemented this analysis using HUES and HUES-derived differentiated cells. Total RNA was extracted and next generation sequencing was performed on polyA-selected transcripts (50nt, paired-end sequencing) and small-RNAs (50nt, signle-end sequencing) on Illumina HiSeq sequencer. More than 1.5 billion reads were generated. Small-RNA-Seq reads were aligned against mature miRNAs, precursors and relevant genomic loci whilst RNA-Seq reads were aligned against the genome using a spliced aligner. Both miRNA and mRNA differential expression analysis was performed using DESEQ and Pathways controlled by significantly differentially expressed miRNAs were identified using DIANA-miRPAth v2.0. Using integrated mRNA-miRNA functional analyses we aim to identify major regulatory networks during differentiation of human neurons and A53T-dysregulated pathways.

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Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: Fundação para a Ciência e Tecnologia Grant SFRH/BD/80884/2011

Title: Deciphering the molecular effects of alpha-synuclein in the nucleus: A new concept in synucleinopathies

Authors: ***R. O. PINHO**[1], L. SOREQ[2], H. SOREQ[3], L. FONSECA[4], M. ZWECKSTETTER[4], K. GOTOVAC[5], F. BOROVEčKI[5], C. REGO[6], L. CORREIA GUEDES[7], J. J FERREIRA[7], T. F OUTEIRO[1]
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Abstract: Alpha-synuclein (aSyn), a common player in both sporadic and familiar forms of Parkinson's Disease (PD), was first described as a pre-synaptic and nuclear protein. While the role of aSyn in the pre-synaptic compartment has been widely studied, both the nuclear localization and the role of the protein in the nucleus are still unclear. Recent efforts revealed that nuclear aSyn binds histones and modulates their acetylation, suggesting a putative effect of aSyn on transcription. Here, we investigated both transcriptional changes in rapid and slow progressive PD patients and the role of aSyn in the nucleus. We analyzed the gene expression profiles of a large cohort of two clinically-distinguishable groups of PD patients, based on the rate of disease progression. By analyzing the transcriptional profiles in blood samples from those patients, we identified a subset of >200 differentially expressed genes whose function is linked to DNA repair, purine/pyrimidine biosynthesis, transcription regulation, ubiquitin-proteasome system and membrane trafficking. Selected genes were further validated by real-time PCR, in a subset of patients' blood cells. Importantly, some of the selected genes were also found to be significantly deregulated in differentiated Lund Human Mesencephalic cells that were either treated with MPP+, or over expressed aSyn. To investigate the presence of aSyn in the nucleus we used cell culture models, and transgenic mice expressing either wild type or A30P mutant aSyn. Our results demonstrated that aSyn is present in nuclear fractions of embryonic stage (E14) and in the midbrain region of adult A30P mice, as well as in human neuroblastoma cells. We also found that oxidative stress promoted the translocation of A30P and E46K aSyn mutants from the cytoplasm into the nucleus of cells 24h after H2O2 treatment. Next, to identify nuclear factors that interact with aSyn, we studied aSyn-DNA interactions by performing NMR experiments, genome-wide chromatin immunoprecipitation (ChIP)-sequencing and dual-luciferase assays. Our preliminary NMR results indicate that aSyn interacted with both DNA fragments and mononucleosomes. ChIP-sequencing experiments showed that aSyn can bind to the promoter regions of several genes, including NEDD4, CDC42 and SLC4A5. Furthermore, dual-luciferase assays confirmed that asyn overexpression increased SLC4A5 promoter activity. Altogether, our results suggest that transcriptional deregulation and nuclear aSyn might be important players in the pathophysiology of PD, and identify a panel of fingerprint genes that may serve as biomarkers in the prognosis of PD progression.

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Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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the Mangurian's Foundation

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Title: Nutrient deprivation induces alpha-synuclein aggregation through ER stress response and SREBP2 pathway

Authors: *P. JIANG, M. GAN, W.-L. LIN, S.-H. YEN
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Abstract: Abnormal accumulation of filamentous α-synuclein (α-syn) in neurons, regarded as Lewy bodies (LBs), are a hallmark of Parkinson disease (PD). Although the exact mechanism(s) underlying LBs formation remains unknown, autophagy and ER stress response have emerged as two important pathways affecting α-syn aggregation. In present study we tested whether cells with the tetracycline-off inducible overexpression of α-syn and accumulating α-syn aggregates can benefit from autophagy activation elicited by limited nutrient deprivation, since this approach was reported to effectively clear cellular polyglutamine aggregates. We found that limited nutrient deprivation of non-induced cells did not affect cell viability, but significantly activated autophagy reflected by increasing the level of autophagy marker LC3-II and autophagic flux and decrease of endogenous α-syn. Cells with induced α-syn expression alone displayed autophagy activation in an α-syn dose-dependent manner to reach a level comparable to that found in non-induced, nutrient deprived counterparts. Nutrient deprivation also activated autophagy further in α-syn induced cells, but the extent was decreased with increase of α-syn induction duration, indicating α-syn overexpression reduces the responsiveness of cells to nutrient deprivation. Moreover, the nutrient deprivation enhanced accumulation of α-syn aggregations concomitant with significant increase of apoptosis as well as ER stress response, SREBP2 activation and cholesterolgenesis. Importantly, the α-syn aggregate accumulation and other effects caused by nutrient deprivation were counteracted by knockdown of SREBP2, treatment with cholesterol lowering agent_lovastatin, or by GRP78 overexpression, which also caused decrease of SREBP2 activity. Similar results were obtained from studies of primary neuronal cultures with α-syn overexpression under nutrient deprivation. Together our findings suggested that down-regulation of SREBP2 activity might be a mean to prevent α-syn aggregates accumulation in PD via reducing cholesterol levels.

Disclosures: P. Jiang: None. **M. Gan:** None. **W. Lin:** None. **S. Yen:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS057656

Title: α-Synuclein disrupts iron import in a yeast model of Parkinson's disease

Authors: *S. N. WITT[1], D. PATEL[2]
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Abstract: We have been using a yeast model of PD to investigate the role of α-synuclein (α-syn) in controlling the intracellular trafficking of two proteins involved in iron uptake. Fet3-Ftr1 complexes, which reside in the plasma membrane, regulate high affinity iron uptake in yeast cells. Fet3 is homologous to ceruloplasmin and hephaestin, which are two human proteins involved in iron transport. A glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed in neurons. Under high iron conditions, Fet3-Ftr1 complexes are targeted to the vacuole/lysosome for degradation. In contrast, under low iron conditions, the Fet3-Ftr1 complexes shuttle back and forth between the plasma membrane and endocytic recycling vesicles. First, we determined that α-syn is extremely toxic to non-dividing stationary-phase yeast cells. The median lifetime of wild-type cells without α-syn is 13.5 ± 5 d and 4.0 ± 1 d with α-syn. In fet3Δ and ftr1Δ deletion strains, which should contain less iron than wild-type cells, the median lifetime is 7.0 ± 1.5 d, and this value does not change with α-syn expression. Second, we performed a colorimetric-based iron assay to determine the cellular iron content. We found that wild-type cells expressing α-syn have approximately 30% less iron than the same cells without α-syn expression; whereas, fet3Δ and ftr1Δ cells have approximately 20% less iron than identically treated wild-type cells. The combined results suggest that α-syn decreases the level of iron in wild-type cells, and the decrease in intracellular iron is responsible in part for the accelerated aging. Fet3 and Ftr1 form binary complexes in the endoplasmic reticulum. Under conditions of low iron, Fet3-Ftr1 complexes partition between the plasma membrane and recycling endocytic vesicles. α-Syn binds to vesicles, and at sufficient concentrations it can disrupt endocytosis. To determine whether α-syn disrupts the endocytic recycling of Fet3-Ftr1 complexes, we used strains with integrated copies of Fet3-GFP or Ftr1-GFP. Under low iron conditions, α-syn significantly decreases the number of Ftr1-GFP molecules on the plasma membrane compared to cells without α-syn. This result is consistent with α-syn disrupting

endocytic recycling of iron transporters, which causes a suboptimal number of Fet3-Ftr1 complexes to reach the plasma membrane. α-Syn thus decreases the amount of intracellular iron. We propose that the combination of decreased intracellular iron and stalled vesicles containing Fet3-Ftr1 complexes trigger cell death. Experiments are planned to test whether α-syn modulates the intracellular trafficking of iron transporters in neurons.

Disclosures: S.N. Witt: None. **D. Patel:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Research Funding for Longevity Sciences (23-2 and 26-23) from National Center for Geriatrics and Gerontology (NCGG)

Title: DHA induced the dysfunction of cellular proteolysis system and the formation of α-synuclein aggregates in SH-SY5Y cells

Authors: *M. SHAMOTO-NAGAI[1], M. NAOI[2], T. OSAWA[2], N. MOTOYAMA[1], M. MINAMIYAMA[1], W. MARUYAMA[1]
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Abstract: Intra-neuronal Lewy body (LB) is the pathological hallmark of Lewy body disease, Parkinson disease (PD), Lewy body dementia (DLB), and pure autonomic failure (PAF). Alpha-Synuclein (αS) is the major component of LB and under physiological conditions plays an important role in maintaining function of synapic vesicle and mitochondria, dopamine production and regulation of cellular redox state. Studies in familial and sporadic PD indicate that accumulation of αS with abnormal conformation is the causative factor. Dysfunction of proteolysis system, including ubiquitin proteasome system (UPS) and autophagy, is closely associated with the accumulation of abnormal proteins. Docosahexaenoic acid (22:6n-3, DHA) is one of the most common long chain polyunsaturated fatty acids (PUFA) and is highly enriched in the membrane of brain and retina. DHA is a potent antioxidant, but it is easily oxidized to produce lipid peroxides. In our previous study, we established human SH-SY5Y cells over-expressing αS (Syn-SH cells). After treatment of Syn-SH cells with DHA, cell death was caused

by mitochondrial dysfunction accompanied with accumulation of αS modified with DHA-derived lipid peroxides. In this paper, we analyzed the mechanism of oxidatively modified αS in concern to proteolysis system. Syn-SH cells were transfected with proteasome sensor vector (Syn-PSV-SH cells) to measure *in situ* proteasome activity. After incubated with 20 μM DHA for 3 days, the accumulation of the fluorescent protein was observed in Syn-PSV-SH cells, indicating the decreased proteasome activity *in situ*. The proteasome enzyme activity was measured using the cytosol fraction and synthetic peptide (Suc-Leu-Leu-Val-Tyr-MCA) as a fluorescent substrate, but the *in vitro* activity was not changed. Western blot analysis showed increased ubiquitinated proteins in Syn-SH cells treated with DHA for 1 day. These results indicated that DHA treatment impaired UPS in Syn-SH cells, but it was not due to the direct inhibition of the proteasome enzyme activity. Next, we examined the autophagy and lysosomal function in Syn-SH cells treated with DHA by fluorescence imaging using each specific fluorescent probes against autophagosome and lysosome. After DHA addition, it was detected that autophagosome increased continuously, even not markedly, and the lysosomal formation was inhibited in Syn-SH cells. These results suggest that DHA reduced the proteasome activity and inhibited the formation of mature autophagosome. The dysfunction of the proteolysis system is suggested to be involved in the accumulation of abnormal αS and cell death by DHA.

Disclosures: **M. Shamoto-Nagai:** None. **M. Naoi:** None. **T. Osawa:** None. **N. Motoyama:** None. **M. Minamiyama:** None. **W. Maruyama:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: KAKEN 25290014

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Title: Differential expression of alpha-synuclein *in vitro* and *in vivo*

Authors: ***K. TAGUCHI**, Y. WATANABE, A. TSUJIMURA, M. TANAKA
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Abstract: α-Synuclein is the major pathological component of synucleinopathies including Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Recent studies have demonstrated that α-synuclein also plays important roles in the release of synaptic vesicles and synaptic membrane recycling in healthy neurons. However, the precise relationship between the pathogenicity and physiological functions of α-synuclein remains to be elucidated. To address this issue, we investigated the subcellular localization of α-synuclein in normal and pathological conditions using primary mouse hippocampal neuronal cultures. While some neurons expressed high levels of α-synuclein in presynaptic boutons and cell bodies, other neurons either did not or only very weakly expressed the protein. These α-synuclein-negative cells were identified as inhibitory neurons by immunostaining with specific antibodies against glutamic acid decarboxylase (GAD), parvalbumin, and somatostatin. In contrast, α-synuclein-positive synapses were colocalized with the excitatory synapse marker vesicular glutamate transporter-1. This expression profile of α-synuclein was conserved in the hippocampus *in vivo*. In addition, we found that while presynaptic α-synuclein colocalizes with synapsin, a marker of presynaptic vesicles, it is not essential for activity-dependent membrane recycling induced by high potassium treatment. Exogenous supply of preformed fibrils generated by recombinant α-synuclein was shown to promote the formation of Lewy body (LB) -like intracellular aggregates involving endogenous α-synuclein. GAD-positive neurons did not form LB-like aggregates following treatment with preformed fibrils, however, exogenous expression of human α-synuclein allowed intracellular aggregate formation in these cells. Therefore, expression level of α-synuclein is one of the critical factors for the aggregate formation. Taken together, these results suggest the presence of a cell-type dependent mechanism for the regulation of the expression of α-synuclein. In other brain regions, the expression analysis is under progress. These findings will provide new insights for understanding the pathological conditions of neurodegenerative disorders including PD and DLB.

Disclosures: K. Taguchi: None. **Y. Watanabe:** None. **A. Tsujimura:** None. **M. Tanaka:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Mememtum: early detection of neurological disorders app

Portal d'Avall SL.

Title: NEDD4 regulation of the pro-apoptotic protein RTP801 in cellular models of Parkinson's disease

Authors: M. CANAL[1], J. ROMANÍ-AUMEDES[1], N. MARTÍN-FLORES[1], V. PÉREZ-FERNÁNDEZ[1], *C. MALAGELADA GRAU[2]
[1]Pathological Anatomy, Pharmacol. and Microbiology, [2]Univ. De Barcelona, Barcelona, Spain

Abstract: RTP801/REDD1 is elevated in cellular and animal models of Parkinson's disease (PD) and in affected neurons of PD patients. RTP801 over expression is sufficient to promote neuron death in cellular models of PD by a mechanism involving repression of mTOR kinase activity. RTP801 is a protein with a very short half-life (2-5 minutes), so its synthesis and degradation must be subject to a very fine-tuned regulation to precisely modulate mTOR pathway. Hence, elucidating which proteins mediate RTP801 degradation would be a stepping-stone to design new therapies to block neurodegeneration. NEDD4 (neural precursor cell-expressed, developmentally down-regulated 4) is one of the most abundant E3 ubiquitin ligases in mammalian neurons. It ubiquitinates proteins targeting them for proteasomal or endosomal/lysosomal degradation or for trafficking. In models of PD, NEDD4 is protective against alpha-synuclein toxicity. Here, we report that NEDD4 poly-ubiquitinated RTP801 in an *in vitro* cell free system. In cellular models NEDD4 and RTP801 interacted physically to each other. Furthermore, also in cellular models, both PD toxin 6-OHDA and the hypoxia inducer CoCl2 decreased levels of NEDD4, along with an increase in RTP801 protein. Interestingly, ectopic expression of NEDD4 protected from 6-OHDA-induced cell death and against ectopic RTP801-induced cell death, whereas the inactive mutant NEDD4-C867S abrogated this protective effect. To sum up, these findings show that NEDD4 is sensitive to oxidative stress associated to PD and that ectopic NEDD4 is protective by enhancing RTP801 degradation. This suggests that NEDD4 loss of function in PD could participate into neuronal death by elevating RTP801.

Disclosures: M. Canal: None. **C. Malagelada Grau:** None. **J. Romaní-Aumedes:** None. **N. Martín-Flores:** None. **V. Pérez-Fernández:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: German Science Foundation (DFG)

Title: Inhibition of the deubiquitinase UCH-L1 leads to autophagy activation and the clearance of alpha-synuclein aggregates in oligodendroglial cells

Authors: *K. PUKAß, C. RICHTER-LANDSBERG
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Abstract: UCH-L1 is an abundant protein in the brain. It is a member of the deubiquitinating enzyme (DUB) subfamily of ubiquitin carboxy-terminal hydrolases (UCHs). UCH-L1 is primarily expressed in neurons where it is required for maintaining the structure and function of synapses. As a monomer it has hydrolase activity for K48-linked ubiquitin chains and as a dimer it has ligase activity for K63 residues of the ubiquitin molecule. UCH-L1 transfers ubiquitin to alpha-synuclein (a-syn), a soluble, natively unfolded protein, which, as we have shown before, is expressed in oligodendrocytes (ODCs). A-syn and UCH-L1 colocalize in the presynaptic terminal. UCH-L1 is involved in the ubiquitination of tubulin and overexpression inhibits microtubule formation. Furthermore, UCH-L1 is detectable in Lewy bodies in Parkinson's disease, where it colocalizes with a-syn. The present study was undertaken to investigate whether UCH-L1 is present in cultured rat brain oligodendrocytes, the myelin forming cells of the CNS. RT-PCR, immunoblot analysis, and indirect immunofluorescence demonstrate that UCH-L1 is expressed in ODCs, increases during culture maturation, and is distributed throughout the cytoplasm and the cell processes. To test whether UCH-L1 is involved in protein aggregate formation, OLN-a-syn-GFP-LC3 cells, an oligodendroglial cell line stably expressing a-syn and GFP-LC3 was used. GFP-LC3 serves as a marker for the autophagic flux. Cells were incubated with the UCH-L1 specific inhibitor LDN-57444 (LDN). Indirect immunofluorescence demonstrates that small a-syn aggregates, which are observable in control cells, are removed by LDN treatment, while GFP-positive autophagic vesicles are increasingly formed. Western blot analysis shows an increase in free GFP and LC3 II, indicating activation of autophagy. Furthermore we tested whether UCH-L1 is a constituent of glial cytoplasmic inclusions (GCIs) in brains of patients with multiple system atrophy (MSA), which represents a neurodegenerative disease classified as a synucleinopathy with ODC pathology. Immunohistochemistry demonstrates that UCH-L1 is present in GCIs and colocalizes with a-syn. To summarize, UCH-

L1 is expressed in ODCs and its inhibition leads to activation of autophagy and the clearance of a-syn aggregates. Its presence in GCIs in MSA brains, which besides a-syn have been described to contain also cytoskeletal proteins and ubiquitin, indicates that it is involved in abnormal ubiquitination and MT formation during pathogenesis.

Disclosures: K. Pukaß: None. **C. Richter-Landsberg:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant K08NS083738

Title: Lysosomal abnormalities in Parkinson's disease brains

Authors: *M. CHENG[1], S. P. SARDI[2], A. CUERVO[3], D. SULZER[1], S.-H. KUO[1]
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Abstract: Mutations in glucocerebrosidase (GBA) not only lead to Gaucher's disease (GD), known as the most common lysosomal storage disorder, but also increase the risk of Parkinson's disease (PD). We analyzed PD patient brains and found that GBA activity is gently reduced in PD/dementia with Lewy bodies (DLB) without GBA mutation cases, while GBA activity is significantly decreased in PD/DLB with severe or mild GBA mutation cases, accompanied by the accumulation of glucosylceramide (GluCer), a substrate of GBA, showing gain of toxicity. In addition, levels of lysosomal proteins LAMP1 and LAMP2A appear to be up-regulated in PD brains with heterozygous GBA mutations, suggesting general lysosomal dysfunction. Furthermore, another PD related protein - alpha-synuclein monomers and dimers are elevated in PD-GBA cases. In summary, our findings demonstrate a strong correlation between GBA mutations and lysosomal abnormalities, and provide insights into disease pathogenesis and potential therapeutic strategies for GD and PD.

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Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Decrease in UCH-L1 protein in the substantia nigra of neurotoxicant-treated mice is associated with reduced numbers of TH-IR neurons expressing UCH-L1

Authors: *B. M. WINNER[1,2], R. E. WELCH[1], Z. A. DERADE[1], K. J. LOOKINGLAND[1,2,3], J. L. GOUDREAU[1,2,3,4]
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Abstract: A significant proportion of the genes implicated in Parkinson disease (PD) are associated with protein degradation pathways. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is one such neuron-specific protein that has traditionally been described as a de-ubiquitinating (DUB) enzyme. UCH-L1 functions to replenish the pool of ubiquitin monomers to be tagged onto defective proteins. The DUB function of UCH-L1 is important for the ability of neurons to degrade misfolded proteins and deficits in misfolded protein processing is a key feature of PD molecular pathogenesis. Following neurotoxicant exposure, differential expression of UCH-L1 has been observed in brain regions containing dopamine (DA) neurons known to be susceptible in PD, nigrostriatal DA (NSDA) neurons and regions known to be resistant in PD, tuberoinfundibular DA (TIDA) neurons. UCH-L1 protein is increased in mice 24 h after an acute 20 mg/kg dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in regions containing TIDA neurons, but not in regions containing NSDA neurons. UCH-L1 protein is decreased in brain regions containing NSDA neurons, suggesting decreased UCH-L1 expression could mechanistically linked with the susceptibility of these DA neuronal populations. That said, it is unclear if UCH-L1 protein levels change specifically within DA neurons with MPTP treatment. We sought to determine if these neurotoxicant-induced changes in UCH-L1 protein occur specifically within DA neurons in the MBH and ventral midbrain. To this end, transgenic mice expressing green fluorescent protein (GFP) driven by a tyrosine hydroxylase (TH) promoter were treated with saline (10 ml/kg) or MPTP (20 mg/kg, s.c.). Mice were anesthetized and perfusion fixed 24 h following treatment. 20 µm frontal brain sections were taken (100 µm intervals) and immunohistochemically stained for UCH-L1 using a Cy3-labelled secondary antibody. ImageJ software was used to count neurons containing Cy3, GFP or both fluorophores in a semi-

automated manner based on particle size and colour/intensity thresholds. The number of TH-GFP expressing neurons in the substantia nigra was unaltered 24 h following MPTP treatment. Decreased numbers of TH-GFP containing neurons immunoreactive for UCH-L1 were observed in the substantia nigra in MPTP-treated mice. Since the overall number of DA neurons appears unchanged in the NSDA neurons 24 h following MPTP exposure, the observed decrease in UCH-L1 immunoreactive TH-GFP neurons suggests that either UCH-L1 expression is decreased or that the turnover of UCH-L1 is increased in the DA neuronal population that is susceptible to neurotoxicant-induced injury and degenerate in PD.

Disclosures: B.M. Winner: None. **R.E. Welch:** None. **Z.A. DeRade:** None. **K.J. Lookingland:** None. **J.L. Goudreau:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 413.17/Q8

Topic: C.03. Parkinson’s Disease

Support: NUS Graduate School for Integrative Sciences and Engineering (NGS)

Genome Institute of Singapore (GIS), Agency for Science, Technology and Research (A*STAR)

Duke-NUS Graduate Medical School

Title: Gene expression profiling to identify Parkinson’s Disease associated transcripts

Authors: *L. LIN[1,2], L. W. STANTON[1,3,4]
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Abstract: Recent studies of Parkinson’s disease (PD) have taken advantage of patient-derived induced pluripotent stem cells (iPSCs) to recapitulate aspects of the disease *in vitro*. These studies have provided snapshots of the disease process in limited genetic backgrounds and have not interrogated transcriptome level changes associated with disease progression. In our study, multiple PD iPS cell lines carrying different genetic background were employed to identify common transcriptional alterations. In neural progenitors and midbrain dopaminergic neurons

(mDAs) derived from iPSCs, expression profiling studies were performed by microarray and RNA-seq. A number of transcripts were consistently differentially expressed among different PD cell lines, as compared with wild-type. RNA-seq results were further analyzed with a focus on the potential involvement of long non-coding RNAs (lncRNAs), which are functional transcripts known to be involved in neurogenesis and neurological disorders. Furthermore, electrophysiological tests and neurotoxicity assays also contribute to our understanding of disease phenotype *in vitro*, serving as a platform for functional study. Together, these data 1) further validate the iPSC model of PD; 2) uncover the abnormally regulated transcripts; 3) indicate an important role for lncRNAs in PD progression.

Disclosures: L. Lin: None. **L.W. Stanton:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Title: Parkinson's disease linked park15 protein affects bmp signaling through proteasome independent nrage ubiquitination

Authors: *K. C. CHUNG[1], J. KANG[1], A. HONG[1], E. IM[1], Y. LEE[1], H. RHIM[2]
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Abstract: Parkinson’s disease (PD) is characterized by progressive midbrain dopaminergic neuron degeneration and the formation of intracellular protein aggregates, referred to as Lewy

bodies. While defective ubiquitin-proteasome system, mitochondrial dysfunction, oxidative stress, and autophagy impairment have been suggested to play some roles in the PD pathogenesis, mutations in at least seven genes have also been individually linked to familial forms of PD. *F-box only protein 7* (*FBXO7;* also known as *PARK15*) gene mutations are closely associated with progression of the autosomal recessive form of familial PD. *FBXO7* encodes a component of Skp1, Cullin, F-box (SCF)-ubiquitin ligase complexes; however, its cellular targets, including substrates and regulators, are not yet clarified. To identify potential substrates of FBXO7, we performed a yeast two-hybrid screen of a human fetal brain library and identified neurotrophin receptor-interacting MAGE protein (NRAGE) as a novel FBXO7-binding partner. NRAGE is highly expressed during early corticogenesis, specifically in neural progenitors of ventricular zone and in differentiating neuroblasts of the cortex, concomitant with the spatial and temporal occurrence of bone morphogenetic protein (BMP)-mediated apoptosis. At the meeting, we will present the data demonstrating that FBXO7 affects BMP4-mediated signaling through proteasome- independent ubiquitination of NRAGE and augments the formation of downstream signaling components.

Disclosures: K.C. Chung: None. **J. Kang:** None. **A. Hong:** None. **E. Im:** None. **Y. Lee:** None. **H. Rhim:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.19/R1

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation

JSPS KAKENHI Grant Number 25670084

MEXT-Supported Program for the Strategic Research Foundation at Private Universities

the Focus 21 project of the New Energy and Industrial Technology Development Organization (NEDO)

Title: Immunostaining of oxidized DJ-1 in human and mouse brain

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Abstract: DJ-1, the product of a causative gene of a familial form of Parkinson's disease (PD), undergoes preferential oxidation of cysteine residue at position 106 (Cys-106) under oxidative stress. Using specific antibodies against Cys-106-oxidized DJ-1, we examined oxidized DJ-1 immunoreactivity (oxDJ-1 IR) in wild type and DJ-1 knockout mouse brain sections as well as human brain sections from cases classified into different Lewy body (LB) stages of PD and PD with dementia. In mouse brain, the oxDJ-1 IR was particularly enriched in the olfactory bulb, cerebellum, cortex, and hippocampus (Fig. 1A). The oxDJ-1 IR was evident in the substantia nigra of the midbrain of mouse and human (Fig. 1A and 1B). In neuromelanin-containing neurons and nerve fibers of the substantia nigra of human brain section, oxDJ-1 IR was prominently observed, and the LBs also showed oxDJ-1 IR (Fig. 2). OxDJ-1 was detected in the astrocytes of the striatum. In addition, neurons and glias in the red nucleus of the midbrain and the inferior olivary nucleus of the medulla oblongata, which is related to the regulation of movement, showed clear oxDJ-1 IR. Our observations suggest the relevance of DJ-1 oxidation to the homeostasis in multiple sites of brain, including neuromelanin-containing neurons of the ubstantia nigra.

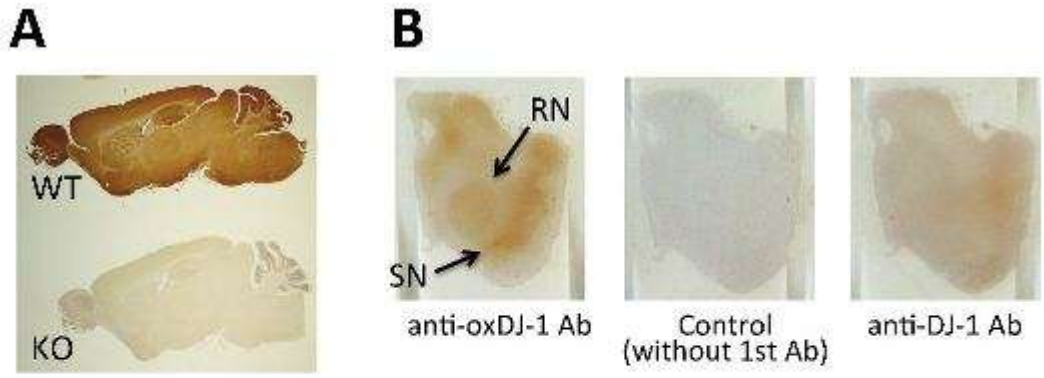

Fig. **1**
A: Sagittal sections from the WT and DJ-1 KO mice brains were stained with anti-oxDJ-1 mAbs.
B: The immunoreactivity of the anti-oxDJ-1 mAb was present throughout the midbrain of human, particularly high levels of oxDJ-1 were observed in the substantia nigra (SN) and red nucleus (RN).

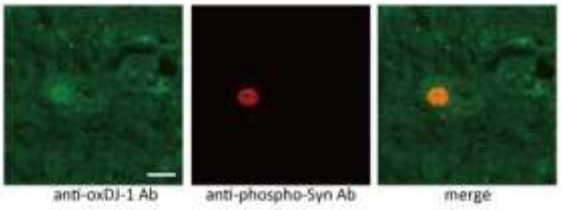

Fig. 2
The areas labeled with oxDJ-1 and phosphorylated α-synuclein (indicative of LBs) antibodies colocalized.

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Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 413.20/R2

Topic: C.03. Parkinson's Disease

Title: Expression and protease activity of mouse legumain are regulated by DJ-1 and secretion of mouse prolegumain into serum is increased in DJ-1-knockout mice

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Abstract: Legumain (EC 3.4.22.34) is an asparaginyl endopeptidase. Legumain is highly expressed in tumors and highly activated in Alzheimer disease brains. Recently, we found that transcription of the legumain gene is regulated by p53 tumor suppressor in HCT116 cells. We and others reported that DJ-1/PARK7, a cancer- and Parkinson's disease-associated protein, works as a coactivator to various transcription factors, including the androgen receptor, p53, PSF, Nrf2, SREBP and RREB1. In this study, we found that expression levels of legumain mRNA and protein and legumain activity were increased in DJ-1-knockout cells. Furthermore, we found that the p53-binding site is present on intron 1 of the mouse legumain gene, that the dot

structure of legumain was increased in DJ-1-knockout cells, and that secretion of prolegumain into the conditional medium and serum was increased in DJ-1-knockout cells and DJ-1-knockout mice, respectively. These results suggest that legumain expression, activation and secretion are regulated by DJ-1.

Disclosures: **T. Yamane:** None. **I. Ohkubo:** None. **H. Ariga:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.21/R3

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS38377

The JPB foundation

Adrienne Helis Grant

Diana Helis Grant

Title: AIMP2 facilitates PINK1 clearance through enhancement of ubiquitin proteasomal degradation

Authors: *S. CHOI[1,2,6], Y. LEE[1,3,2,7], D. KIM[1,2,6], S. KWON[1,9], T. KAM[1,2], S. YUN[1,2,7], G. JUNG[9], D. A. STEVENS[1,2,4], S.-U. KANG[1,2,7], V. L. DAWSON[1,3,2,4,7], T. M. DAWSON[1,2,5,4,8], H. KO[1,2,6]
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Abstract: Gene mutations in PINK1, a mitochondrial serine/threonine kinase, result in autosomal recessive Parkinson's disease (PD). The tight regulation of PINK1 levels in the mitochondria has been proposed to play an important role in mitochondria quality control via mitophagy. However, the mechanisms by which the steady state level of PINK1 are regulated, are poorly understood. Here we show that the parkin substrate, AIMP2, functions as a novel

regulator for PINK1 ubiquitin-proteasome dependent degradation. AIMP2 interacts with both PINK1 and the 26S proteasome subunits to promote the ubiquitin proteasomal degradation of PINK1. Overexpression of AIMP2 decreases PINK1 levels, whereas depletion of AIMP2 increases PINK1 levels both *in vitro* and *in vivo*. Moreover, AIMP2-mediated PINK1 regulation leads to differential kinetics of parkin recruitment to CCCP-damaged mitochondria. These results suggest that AIMP2 serves as a key regulator of PINK1 expression and functions in a mechanism to identify damaged mitochondria for autophagic removal through regulation of PINK1 steady state levels.

Disclosures: S. Choi: None. **Y. Lee:** None. **D. Kim:** None. **S. Kwon:** None. **T. Kam:** None. **S. Yun:** None. **G. Jung:** None. **D.A. Stevens:** None. **S. Kang:** None. **V.L. Dawson:** None. **T.M. Dawson:** None. **H. Ko:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 413.22/R4

Topic: C.03. Parkinson’s Disease

Support: CIHR MOP 119347

Title: Parkinsonism, mutant VPS35, and novel retromer functions in neurons

Authors: *L. N. MUNSIE[1], A. MILNERWOOD[2], P. SIEBLER[3], D. BECCANO-KELLY[1], M. VOLTA[1], C. KLEIN[3], M. FARRER[1]
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Abstract: Vacuolar protein sorting 35 (VPS35) is a core component of the retromer complex, crucial to endosomal protein sorting and intracellular trafficking. Little is known about retromer function in neurons but we recently linked a VPS35 mutation (p.D620N) to familial parkinsonism. Here we show that VPS35 is involved in the trafficking of excitatory AMPA-type neurotransmitter receptors (AMPARs). We examined the affect of the p.D620N mutation on neuronal function. While some measures are unaffected by the p.D620N mutation, we found altered intracellular trafficking and subcellular localization of mutant VPS35, in addition to altered synaptic transmission, AMPAR surface expression, and AMPAR recycling. Perturbations

to synaptic function in the presence of the p.D620N mutation may produce chronic pathophysiological stress and subsequent neurodegeneration

Disclosures: **L.N. Munsie:** None. **A. Milnerwood:** None. **D. Beccano-Kelly:** None. **M. Volta:** None. **M. Farrer:** None. **P. Siebler:** None. **C. Klein:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 413.23/R5

Topic: C.03. Parkinson's Disease

Title: Differential glutamate release from synaptoneurosomes prepared from Parkinson's and Huntington's disease mouse models

Authors: ***J. B. WATSON**[1], C. C. FERNANDEZ[1], E. MASLIAH[2], T. A. SARAFIAN[1]
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Abstract: Alterations in glutamate release have been implicated in the synaptic pathology of multiple neurodegenerative diseases including Parkinson's Disease (PD) and Huntington's Disease (HD). Here a micro-method for preparing synaptoneurosomes (SN) enriched in presynaptic and postsynaptic elements [see JW Chang et al (2012) Neurosci Methods 211:289-295] was used to examine glutamate release from SNs prepared from both an α-synuclein overexpressing PD mouse model (ASOTg or Thy-1 αSyn) and an HD mouse model with expanded glutamine repeats in the mutant huntingtin protein (R6/2 BHD/150). Initially a glutamate oxidase assay coupled to Amplex Red® fluorimetry (Life Technologies/Invitrogen) was optimized over 30 minutes in 96 well plates containing glutamate standards either alone or in the presence of SNs prepared from freshly dissected wildtype (WT) mouse forebrain. SNs receiving multiple drug treatments (4 mM EGTA, 40 mM KCl ± 2 mM $CaCl_2$ or 100 μM $CdCl_2$) confirmed both depolarization- and Ca^{2+}-dependent glutamate release from WT control SNs. Inclusion of TBOA (50 μM) confirmed functional glutamate transporters for synaptic reuptake. Glutamate release (KCl/Ca^{2+}-dependent, mean±SEM pmole/μg protein) was subsequently measured in fresh cortex and striatum SNs prepared from both PD and HD mouse models. Only SNs prepared from ASOTg cortex (male, 2-4 months) showed an increase in glutamate release relative to WT controls (P=0.027). Conversely glutamate release from R6/2 striatum SNs (60-65 days old) was decreased versus WT controls (P=0.033). Future experiments will utilize both

pharmacological treatments and recombinant protein manipulations of PD and HD mouse model SNs to sort out the relevant presynaptic signaling pathways that mediate altered glutamate release.

Disclosures: J.B. Watson: None. **C.C. Fernandez:** None. **E. Masliah:** None. **T.A. Sarafian:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.24/R6

Topic: C.03. Parkinson's Disease

Support: UNAM-PAPIIT IN215114

UNAM-PAPIIT IA202214

Title: Is the presence of perforated synapses after dopamine depletion a sign of maladaptive brain plasticity?

Authors: *M. AVILA-COSTA[1], V. ANAYA-MARTÍNEZ[1], A. GUTIERREZ-VALDEZ[1], J. ORDOÑEZ-LIBRADO[1], J. SANCHEZ-BETANCOURT[1], E. MONTIEL-FLORES[1], J. ESPINOSA-VILLANUEVA[1], P. ALEY-MEDINA[1], L. REYNOSO-ERAZO[2], J. MACHADO-SALAS[2]
[1]UNAM, Neuromorphology Lab., Tlalnepantla Edo Mex, Mexico; [2]UNAM, Tlalnepantla Edo Mex, Mexico

Abstract: Synaptic plasticity is the process by which long-lasting changes take place at synaptic connections. The phenomenon itself is complex and can involve many levels of organization. Some authors separate forms into adaptations that have positive or negative consequences for the individual. It has been hypothesized that an increase in the number of synapses may represent a structural basis for the enduring expression of synaptic plasticity during some events that involve memory and learning; also, it has been suggested that perforated synapses increase in number after some disease and experimental situations. The aim of this study was to analyze whether dopamine depletion induces changes in the synaptology of the striatum of rats after the unilateral injection of 6-OHDA. The findings suggest that after the lesion, both contralateral and ipsilateral striata exhibit an increased length of the synaptic ending in ipsilateral (since 3rd day) and

contralateral striatum (since day 20), loss of axospinous synapses in ipsilateral striatum and a significant increment in the number of perforated synapses, suggesting brain plasticity that might be deleterious for the spines, because this type of synaptic contacts are presumably excitatory, and in the absence of the modulatory effects of dopamine, the neuron could die by excitotoxic mechanisms. Thus, we can conclude that the presence of perforated synapses after striatal dopamine depletion might be a form of maladaptive synaptic plasticity.

Disclosures: **M. Avila-Costa:** None. **V. Anaya-Martínez:** None. **A. Gutierrez-Valdez:** None. **J. Ordoñez-Librado:** None. **J. Sanchez-Betancourt:** None. **E. Montiel-Flores:** None. **J. Espinosa-Villanueva:** None. **P. Aley-Medina:** None. **L. Reynoso-Erazo:** None. **J. Machado-Salas:** None.

Poster

413. Proteopathic Mechanisms in Parkinson's Disease

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Program#/Poster#: 413.25/R7

Topic: C.03. Parkinson’s Disease

Support: UNAM-PAPIIT-DGAPAIN215114

UNAM-PAPIIT-DGAPAIA202214-2

Title: Gender differences on the chronic effect of L-dopa and melatonin in the motor performance in a Parkinson disease model

Authors: ***A. GUTIERREZ VALDEZ**, V. ANAYA-MARTINEZ, J. T. SÁNCHEZ BETANCOURT, J. L. ORDOÑEZ-LIBRADO, E. MONTIEL-FLORES, J. ESPINOSA-VILLANUEVA, S. A. SÁNCHEZ -SORIA, A. TRUJILLO-MARTÍNEZ, F. HUERTA-OLIVAREZ, T. IBARRA-GUTIÉRREZ, M. R. AVILA-COSTA
UNAM, TLALNEPANTLA, Mexico

Abstract: Parkinson disease is a neurodegenerative disorder that affects movement due to the progressive degeneration of dopaminergic neurons of the Substantia nigra pars compacta (SNc). In the last years, it has been observed a greater incidence in men than in women, setting out that this difference is due to the action of sexual hormones. L-dopa is the most common drug for the treatment of PD because of its clinical efficacy; however, long-term L-dopa treatment induces involuntary abnormal movements, several evidences suggest that L-dopa can increase the

oxidative stress pre-existing condition, for that reason we decided to add melatonin as a potent antioxidant. We conducted this study to determine the ability of melatonin alone or in combination with L-dopa to protect nigro-striatal dopaminergic loss induced by 6-OHDA, comparing the results with L-DOPA-only treated rats, as well as determine differences between genders. The experiments were carried out in male, intact female (with estrogen) and ovariectomized (ovx) female rats underwent 6-OHDA-induced lesion in the medial forebrain bundle. The drugs were administered orally daily for 6 months; the motor behaviors were assessed by skilled forelimb use in a staircase test and stepping ability while walking on beams. At the cellular level, the numbers of TH-positive immune cells were evaluated. Surprisingly our results show that independently of the sex, the rats treated with the co-administration of L-dopa/melatonin had the best performance in the motor tasks and increase in the number of dopaminergic cells, which in turn imply the well-preserved synaptology of a less denervated striatum. In addition, we observed differences between sex, showing that intact female rats treated with L-dopa or melatonin had a significantly better recovery than male and ovx female and exhibited conservation of dopaminergic cells, showing that estrogens have neuroprotective effect. Our results suggest that co-administration of L-dopa/melatonin could be a possible candidate for the treatment of PD to prevent or slow the damage caused by L-dopa treatment. Also, the estrogens may exert beneficial effect against the development and progression of the disease and this could be used like in adjunct treatment of PD.

Disclosures: A. Gutierrez Valdez: None. **V. Anaya-Martinez:** None. **J.T. Sánchez Betancourt:** None. **J.L. Ordoñez-Librado:** None. **E. Montiel-Flores:** None. **J. Espinosa-Villanueva:** None. **S.A. Sánchez -Soria:** None. **A. Trujillo-Martínez:** None. **F. Huerta-Olivarez:** None. **T. Ibarra-Gutiérrez:** None. **M.R. Avila-Costa:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 414.01/R8

Topic: C.03. Parkinson’s Disease

Support: JSPS KAKENHI grant number 24591304

Title: The effect of electrical stimulation of the subthalamic nucleus or internal part of the globus pallidus to primate striatal interneurons

Authors: *Y. SHIMO[1], A. NAKAJIMA[1], T. UKA[2], N. HATTORI[1]
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Abstract: Background: Deep brain stimulation (DBS) of the subthalamic nucleuse (STN) or internal segment of the globus pallidus (GPi) is an established therapy for advanced motor symptoms in Parkinson's disease (PD). Previous studies showed that stimulation of the STN or GPi affects GPi neuron or thalamic neuron activity, however, the effect on the striatal neuron activity by STN or GPi DBS is still unclear.To investigate the effect of STN or GPi stimulation on the activity of striatal neurons, we recorded neuronal activity of the interneurons of primate putamen during high-frequency electrical stimulation of STN or GPi. Methods: Neuronal activity of the interneurons in the putamen (tonically active neurons: TANs) was recorded extracellularly during electrical stimulation of the STN or GPi (frequency: 130Hz, pulse width 60 micro seconds, stimulus current 0.4mA or 0.1mA for 30 seconds) in two normal monkeys. TANs were identified by a characteristic firing pattern (Shimo and Hikosaka 2001). Results: We examined 72 TANs activity during STN (55 neurons) or GPi (17 neurons) stimulation. Many TANs reduced their activity during STN or GPi stimulation (88% of recorded neurons during STN stimulation, 74% of recorded neurons in GPi stimulation, $p<0.05$ Mann-Whitney U test). Inhibitory responses of TANs during STN stimulation were diminished after local injection of sulpiride (10 μg/μl, total 1.2 μl). Moreover, many of the GPi neurons were inhibited by STN stimulation (11/17 neurons, $p<0.05$ Mann-Whitney U test). Conclusion: TANs are considered to be cholinergic interneurons. This study showed that electrical stimulation of the STN or GPi influences the activity of neurons which are located in the upstream structure of the basal ganglia, and dopamine may play an important role in the response. These results indicate that modulating of TANs activity may be one possible therapeutic mechanism of the STN or GPi DBS.

Disclosures: Y. Shimo: None. **A. Nakajima:** None. **T. Uka:** None. **N. Hattori:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 414.02/R9

Topic: C.03. Parkinson's Disease

Support: Health Research Council of New Zealand

Neurological Foundation of New Zealand

Title: Physiological patterns of optogenetic stimulation in the motor thalamus restore reaching in parkinsonian rats

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Abstract: High frequency deep brain stimulation (DBS) of the subthalamic nucleus or globus pallidus internus is a standard treatment option in advanced Parkinson's disease (PD) to reduce akinesia and rigidity, whereas DBS and thalamotomy of the motor thalamus (Mthal) improves tremor. The primary aim of this study was to investigate if there are better ways to stimulate the Mthal to improve akinesia. Specifically, we explored if neural activity previously recorded in the Mthal of a behaving control rat and played into the brain of parkinsonian rats might restore reaching movements. We used a chronically implanted fibreoptic probe in the Mthal of adult male Wistar rats to selectively stimulate glutamatergic neurons transduced with channelrhodopsin-2 by injection of lentiviral vector (pLenti.CamKII.hChR2(H134R).mCherry). Rats performed a reach-to-grasp task for food pellets, homologous to human reaching, and we recorded the number of reaches per 5 min before, during and after optogenetic stimulation. We induced acute parkinsonism by injecting the dopamine antagonist haloperidol (0.03-0.07 mg/kg, s.c.) so each rat could act as its own control (vehicle injection). Control rats typically executed over 150 reaches per 5 min period, whereas parkinsonian rats displayed marked akinesia, executing less than 20 reaches per 5 min period. We compared the effect of DBS using complex physiological patterns previously recorded in the Mthal during reaching behaviour, with tonic DBS delivering the same number of stimuli per unit time (rate-control 6.2 Hz) and with stimulation patterns commonly used for DBS in other brain regions (tonic 130 Hz, continuous theta burst (cTBS), and tonic 15 Hz rate-control for TBS). Blue light (473 nm) stimulation with the physiological reaching pattern significantly restored reaching in parkinsonian rats (two-way ANOVA; time $p = 0.0095$; pattern $p = 0.0092$), which was dependent on pattern and not rate as tonic 6.2 Hz stimulation did not improve reaching. In addition, standard patterns of stimulation failed to improve reaching in parkinsonian rats, although cTBS showed a strong trend ($p = 0.0562$) to increase the number of executed reaches. In contrast, blue light stimulation did not alter reaching in control rats for any of the patterns (time $p = 0.1496$; pattern $p = 0.3906$). Additionally, stimulation with yellow light (561 nm) did not alter reaching performance in control or parkinsonian experiments for any of the stimulation patterns (all: $p > 0.19$). These data indicate that the pattern of stimulation in the Mthal is critical to improve reaching in parkinsonian rats and that Mthal may be an effective site to treat the akinesia of parkinsonism.

Disclosures: **S. Seeger-Armbruster:** None. **C. Bosch-Bouju:** None. **S.T.C. Little:** None. **R.A. Smither:** None. **S.M. Hughes:** None. **B.I. Hyland:** None. **L.C. Parr-Brownlie:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

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Program#/Poster#: 414.03/R10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ministère de l'Enseignement supérieur et de la Recherche

Fondation de France

Title: Optogenetics mapping of the dynamic properties of the Hyperdirect and Indirect Pathways

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Abstract: The cortico-basal-ganglia (CBG) loop is a complex network involved in action selection and decision making. The appropriate control of CBG's dynamic states is necessary to ensure normal locomotion as attested by the emergence of hyper-synchronized β-oscillations condition in Parkinson's disease (PD). The origin of exacerbated β-oscillations (15-30 Hz) is correlated with the degree of akinesia/bradykinesia which is one of the major symptoms of PD. For now, the emergence and the maintenance of these exacerbated oscillations in motor CBG are still debated. However some computational studies proposed a role of the hyperdirect pathways and Globus pallidus-Subthalamic nucleus (GP-STN) network on the establishment of this state. By receiving GP and motor cortex (mCx) projections, STN forms a bottleneck between two major pathways of CBG (indirect and hyperdirect pathways). Thus the STN is an appropriate target to manipulate the dynamic of CBG loops. The goal of this study is to unravel the role of the mCx and GP inputs in the emergence and the maintenance of β-oscillation using optogenetic and electrophysiological approaches. We used optogenetics toolboxes to selectively modulate GP and mCx neurons and study their involvement in the emergence/maintenance of β-oscillation in controls or parkinsonian rat. Our electrophysiological data show how selective manipulation of mCx and GP inputs impact on the maintenance, the genesis and the propagation of β-oscillation in the CBG loop.

Disclosures: B. De La Crompe: None. **N. Mallet:** None. **F. Gonon:** None. **T. Boraud:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Program#/Poster#: 414.04/R11

Topic: C.03. Parkinson's Disease

Title: mGRASP-assisted synaptic mapping of external globus pallidus (GPe) - subthalamic nucleus (STN) circuits in health and Parkinson's disease model

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by motor dysfunction resulting from dopamine deficiency-induced functional changes in basal ganglia networks. The basal ganglia, which are composed of multiple subcortical nuclei, are known to regulate movements and emotions through a precise balance of direct and indirect pathways. Within the basal ganglia, a key circuit is the reciprocal network between the GPe and the STN. Deep brain stimulation (DBS) of STN has been shown to relieve symptoms of the patients with advanced PD and is considered to be an effective treatment for various neurological and psychotic diseases including movement disorders. However, the mechanism underlying the effects of DBS remains unknown, and even fundamental characteristics such as the functional anatomy and synaptic profiles of these circuits are unclear. In fact, the complexity of neuronal circuit organization in the GPe-STN network (such as its heterogeneous neuronal population and complex patterns of cell type-specific convergence and divergence) has long been underappreciated. In this study, we perform a fine-scale synaptic mapping of the GPe-STN connection using a newly developed synapse labeling tool, mammalian GFP reconstitution across synaptic partners (mGRASP). In MPTP-induced PD model, we observed a significant decrease in tyrosine hydroxylase (TH)-positive neurons in SN, which resulted in a significant decrease in TH-positive axonal projections in the striatum, and notable PD-like motor dysfunction assessed by the vertical grid test. To dissect cell-type specific synaptic mappings, we then introduced presynaptic mGRASP in parvalbumin (PV)-positive neurons of the GPe and postsynaptic mGRASP in the STN of the PD mouse model. Our preliminary results suggest that mGRASP fluorescence signals indicating the presence of

synapses were evident on the soma and dendrites of STN neurons and further that synapses are decreased in the PD mouse model. We are presently conducting detailed analyses of synaptic changes together with a fundamental characterization of the GPe-STN network in healthy and PD model mice. A clear understanding of connectivity characteristics associated with PD will guide us to understand the relationship between these circuits and behavior, mechanisms of DBS effects, and to develop more effective treatments for the patients with PD.

Disclosures: K. Osung: None. **H. Park:** None. **B. Lee:** None. **S. Druckmann:** None. **L. Feng:** None. **W. Oh:** None. **S. Paek:** None. **J. Kim:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: This study was supported by IU-CRG.

Title: Correlation of synchronized dynamics in cortical and basal ganglia networks in Parkinson's disease

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Abstract: Increased oscillatory power and synchrony in the beta band in cortico-basal ganglia circuits is well described in patients with Parkinson's disease. The synchrony between basal ganglia and the cortex has been observed and described. Synchronized dynamics within cortical networks and within the basal ganglia has being studied too (such as synchronization between spikes and Local Field Potentials, LFPs). Given the presumed relevance of both cortical and basal ganglia neurodynamics to the symptoms of Parkinson's disease, it is interesting to explore not only synchrony between cortex and basal ganglia, but also the correlations between synchronous dynamics within the cortical networks and in the basal ganglia networks. In the present study, we consider correlations of cortical and basal ganglia synchronized dynamics at the beta band in parkinsonian patients. We record neuronal unit (spikes) and LFP from subthalamic nucleus (STN) simultaneously with scalp EEGs recordings from motor and

prefrontal areas during microelecrtrode-guided implantation of deep brain stimulation (DBS) electrodes. We then analyze the correlation of the phase synchronies between the synchronous activity of spike-LFP pairs and the synchronous activity between EEG electrode pairs. We first study the phase synchrony during relatively short temporal running windows, and then study the temporal correlations between these synchronous activities to explore the temporal correlations of cortical and basal ganglia networks at the beta band. We found that intrahemispheric phase synchronization between motor EEG electrode is significantly correlated (with some time-delay) with the spiking unit-LFP phase synchronization as they develop in time. This suggests that the change in the degree of synchrony in cortical motor networks is related with (and, perhaps, induces) the change in the coherence of oscillatory interactions within basal ganglia circuits. The correlations between dynamic activities in cortical and subcortical networks points to the complex interconnectedness of the cortical and subcortical circuits in the pathophysiology of Parkinson's disease and signifies further study of cortico-subcortical interactions on different time-scales. We also consider a predictive aspect of the relationship between synchronization of EEG oscillations (which can be obtained noninvasively) and spiking unit-LFP synchrony in STN (which may be recorded only during invasive surgical procedures).

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: Erasmus Mundus Doctorate Programme

Title: Increased interhemispherical synchronization in the hemiparkinsonian rat: A multielectrode approach

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Abstract: Increased synchronization patterns are detected in a wide range of cortical regions and basal ganglia in parkinsonism. However, it is not known how dopamine depletion in basal ganglia affects different cortical areas, their synchronization to each other and to basal ganglia. In the current study, we performed multielectrode recordings in a freely moving rat model of parkinsonism to explore local and interregional changes. Local field potentials were recorded from 12 cortical (motor and sensory areas) and 2 striatal (sensori-motor sector) sites. Serial recordings were performed of behaving rats before and after a unilateral 6-hydroxydopamine injection into the medial forebrain bundle. We computed spectral power and interregional synchronization (Phase Lag Index) before and after the dopaminergic cell lesion in the substantia nigra. Our recordings revealed an elevated beta peak in the power spectrum solely in the motor-related brain regions of the lesioned hemisphere, viz. motor cortical areas and striatum. Moreover, these areas showed enhanced coupling in the beta band compared to the pre-lesion condition. Interhemispherical synchronization was increased between motor cortical areas in the two hemispheres. Cortico-striatal beta synchronization was increased between striatum in the lesioned hemisphere and multiple cortical areas (on both sides of the brain). The contralateral striatum did not show such changes. Our findings indicate that the investigated cortico-striatal system shows widespread synchronization changes in response to the dopaminergic cell depletion in substantia nigra. These changes involve both hemispheres, unlike the observed power spectral alterations.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: Ministry of Education, Culture, Sports, Science and Technology, Japan, 24591306

Title: Disrupted connectivity of motor loops within the basal ganglia in atypical parkinsonism

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Abstract: Atypical Parkinsonism (AP) reportedly includes altered connectivity of neural loops involving the basal ganglia. Little is known of any changes in the connectivity of motor loops. The goal of this study was to further understand the connectivity within the basal ganglia-thalamo-motor (BGTM) loop in AP. Eight AP patients and eight patients with Parkinson disease (PD) performed a protocol involving self-initiated (SI) finger movements while being scanned with functional magnetic resonance imaging (fMRI). In the mapping, there was no difference between the groups. Analysis using structural equation modeling (SEM) revealed significant positive interactions within the right BGTM loop during the SI task. PD patients showed reduced connectivity from the thalamus to the motor cortices, while there was decreased connectivity within the basal ganglia in the AP group, which is the first demonstration of this phenomenon. These results suggest different pathophysiology of the two entities involving different loci in the BGTM loop, and illustrate the utility of SEM for network analysis.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: German Research Foundation in the Clinical Research Group 219

Title: Electrophysiological mapping of alpha and beta local field potential activity across the motor thalamus: Comparison to *in vivo* cerebello-pallidal fibre distribution in humans

Authors: E. PELZER[1], K. A. M. PAULS[2], N. BRAUN[2], M. MAAROUF[3], S. HUNSCHE[3], L. TIMMERMANN[2], *M. TITTGEMEYER[1]
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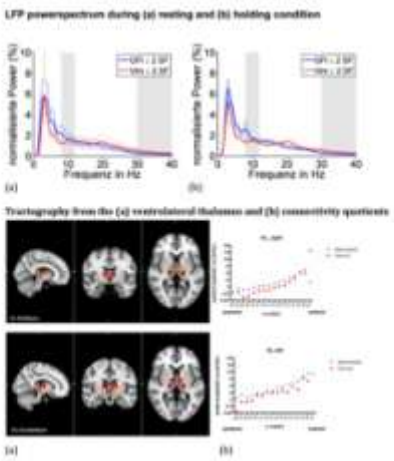
Abstract: Neural oscillations are thought to underlie coupling of spatially remote neurons and gating of information within the human sensorimotor system. Different frequency ranges have been associated with different movements and neurological diseases. In dystonia, a prominent low frequency peak has been found at rest in the globus pallidus (GP). Little is known about neural oscillations at the network level in other subcortical parts of the motor system. The

thalamus is particularly interesting since it links subcortical structures like the cerebellum and the GP with cortex, and is known from tracing studies in animals to show differential connectivity to these structures. Thus, we here investigated the electrophysiological profile of the ventrolateral thalamus in dystonic patients undergoing surgery for deep brain stimulation at rest and during voluntary movement; we compared these findings to tractography-based connectivity distributions of cerebellar and pallidal projections in healthy human subjects. Local field potential (LFP) activity in the beta frequency band significantly increased from anterodorsal to posteroventral direction ($p < 0.05$), while alpha activity decreased -i.e. posteroventral areas showed less alpha and more beta activity than more anterodorsal areas. Regarding tractography results, posterior regions were significantly represented by dentate projections; more anterior regions, however, showed a stronger pallidal connectivity. Electrophysiological recordings and connectivity analysis had both a territory specific accentuation within the motor thalamus. These findings let to assume that the basal-ganglia-thalamo-cortical loops are not only anatomically separated from the cerebello-thalamo-cortical loop, but also functionally segregated through distinct oscillatory frequencies. Further studies are needed to elucidate the exact nature of this anatomical-functional relationship and their interaction.



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Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

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Program#/Poster#: 414.09/S4

Topic: C.03. Parkinson's Disease

Title: Investigation of subthalamic and peripheral motor unit activity in Parkinson disease during an isometric grip task

Authors: *K. G. HAMMOND[1], C. L. GONZALEZ[2], H. C. WALKER[2]
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Abstract: Relatively little is known about how subthalamic activity is modulated by movement in awake, behaving humans with idiopathic Parkinson disease (PD). METHODS: In patients undergoing subthalamic deep brain stimulation for routine care, we evaluated single unit discharges in the hand region of the subthalamic nucleus with parallel measurement of peripheral motor unit activity at five different levels of submaximal isometric grip force. RESULTS: We studied 22 neurons from 6 PD subjects, and participants readily performed the behavioral task. Subthalamic units showed heterogeneous responses, with individual units showing increased activity, decreased activity, or selective responses during the dynamic versus the static phase of the isometric force. At the group level, the discharge frequency of subthalamic units increased during the dynamic but not the static component of the grip force ($p=0.001$, pairwise comparison $p=0.03$, repeated measures ANOVA). With increasing levels of static force, there were corresponding increases in motor unit activity measured by both surface and intramuscular EMG, yet there was no corresponding change in subthalamic nucleus discharge rate at our level of statistical power ($p=0.83$). CONCLUSIONS: During an isometric grip task, subthalamic hand units in humans with PD discharge preferentially during dynamic versus static force conditions. Subthalamic activity did not decrease with increasing levels of static force. Better defining the role of the subthalamic region in specific motor behaviors could improve our understanding of basal ganglia physiology and ultimately guide new approaches to therapy.

Disclosures: K.G. Hammond: None. **C.L. Gonzalez:** None. **H.C. Walker:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Program#/Poster#: 414.10/S5

Topic: C.03. Parkinson's Disease

Title: Role of pedunculopontine cholinergic neurons in gait

Authors: *N. FARHANI[1], R. RAJAKUMAR[2], M. S. JOG[3]
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Abstract: Parkinson's disease (PD) patients suffer from tremor, rigidity and bradykinesia, and these symptoms are associated with severe dopaminergic nigrostriatal denervation. These symptoms are responsive to L-DOPA treatment and/or deep brain stimulation. In advanced stages of PD, other symptoms that are unresponsive to these treatments, including gait and balance abnormalities and impaired control of complex movements, emerge. The later symptoms are not prominent at early stage of PD when there is a significant dopaminergic neuronal loss and they are also not responsive to levodopa. Therefore, the postural and gait abnormalities are believed to be not a consequence of dopaminergic cell loss. Pedunculopontine nucleus (PPN) is located in the caudal midbrain and contains neurons possessing either acetylcholine or GABA, dispersed throughout the nucleus. PPN sends direct projections to the groups of the reticulospinal neurons that in turn send descending commands to spinal networks referred to as central pattern generator, which is involved in synergistic activation of muscles responsible for gait and locomotion. PPN also sends cholinergic projections to a group of neurons in the brainstem that in turn send projections to the reticulospinal neurons and regulate their activity. Determining topographical organization of cholinergic neurons within the PPN that are involved in different aspects of gait and efferent circuitry involved in mediating PPN's role in gait may facilitate in studying gait abnormalities in PD. In the present study, an anterograde neuronal tracer (e.g., BDA) that labels the axonal projection sites of PPN neurons are microinjected into each of the topographical areas of the PPN, and target sites of cholinergic neurons that mediate different aspects of gait will be determined. The same rats will also receive a retrograde tracer (e.g., Flurogold) into the lumbar spinal cord to label reticulospinal neurons projecting to the lumber spinal segments. Sections will then be immunolabeled for vesicular choline transporter, GAD67 or VgluT2 to identify cholinergic terminals. Those neurons containing Flurogold and receiving cholinergic terminals from the PPN will be deemed as the possible neurons involved in mediating efferent circuitry involved in mediating PPN's role in controlling lumbar spinal neurons. Our results will identify the topographical areas and neuronal circuitry and future pharmacological studies to identify the role of PPN in gait and locomotion.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Title: Cortical synchronization is altered in freezers with Parkinson's disease during dual task interference

Authors: *M. A. SCHOLTEN[1,2,3], R. GOVINDAN[4], C. BRAUN[5], C. PLEWNIA[6], A. GHARABAGHI[7], R. KRUEGER[1], D. WEISS[1]
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Abstract: Introduction The pathophysiological basis of freezing of gait (FOG) in Parkinson's disease (PD) is incompletely understood. Cortical processing conflicts may play an important role in provoking FOG for example, when gait is interfered with a cognitive task (dual tasking). Freezing phenomena are not restricted to gait but may also occur during repetitive finger movement. In this study, we characterized the cortical correlates of dual tasking during repetitive finger movement in healthy controls (HC) and gait freezers. Methods We included 12 PD patients with STN-DBS and FOG (9 male, age 60.5 ± 12.6 years) and 12 age- and sex matched healthy controls (7 male, age 61.9 ± 10.1 years). They were instructed to perform internally generated continuous tapping with the right index finger with and without dual task. The interference task was a phonemic verbal fluency task. Subsequently, we analyzed the activity and synchronization (coherence) of the cortex by calculating the corticocortical coherence and phase synchronization index. Results The performance of the interference task did not differ between healthy controls and PD patients. The cortical activity did not change during single and dual task in both healthy controls and PD patients. Healthy controls showed alpha band synchronization in the bilateral centro-parietal areas in the single task that desychronized during dual tasking. PD patients showed similar synchronization in both single- and dual task. Instead, healthy controls demonstrated an increase of synchronization in the frontal areas during dual tasking unlike PD patients. Discussion PD patients demonstrate during dual tasking i) absent desynchronization in the bilateral centro-parietal areas and ii) absence of synchronization in the frontal areas. Abnormal fronto-centro-parietal synchronization patterns may parallel the cortical processing deficits in gait freezers.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Title: Dopamine depletion results in frequency-dependent disinhibition of corticostriatal transmission *in vivo*: Role of local GABAergic function

Authors: *V. R. JAYASINGHE[1], A. R. WEST[2], K. Y. TSENG[3]
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Abstract: Parkinson's disease is strongly associated with the emergence of increased beta oscillations within the basal ganglia, which is thought to reflect a disruption of corticostriatal transmission, and thereby exacerbate motor deficits associated with this disease. The mechanisms underlying the elevated synchronization at beta frequencies observed following dopamine lesion remain unclear. However, dysfunction of local GABAergic interneurons may play a key role as these cells provide robust feed-forward inhibition to striatal projection neurons, and this inhibition is impaired in the dopamine-depleted, parkinsonian striatum. Therefore, the goal of this study was to determine if impaired GABAergic function could underlie the increased synchrony within the corticostriatal pathway at specific frequencies observed in the parkinsonian state. *In vivo* electrophysiological recordings performed in the dorsal striatum of rats revealed a progressive increase in the inhibitory modulation of the local field response evoked via motor cortex train stimulation at 10 (alpha), 20 (beta), and 40 Hz (gamma). Interestingly, this frequency-dependent striatal inhibition evoked during cortical stimulation was lacking in the dopamine-depleted striatum of rats that received the unilateral 6-OHDA lesion. We next asked whether local GABAergic transmission controls striatal responsivity to cortical drive in a frequency dependent manner. Local infusion of the GABA-AR antagonist picrotoxin into the dopamine-intact striatum revealed a frequency-dependent disinhibition of corticostriatal transmission which was similar to that seen in the dopamine-

depleted striatum. Importantly, infusion of picrotoxin into the dopamine-depleted striatum failed to further disrupt the disinhibitory corticostriatal response. Together, these results highlight the possibility of an impaired striatal GABAergic inhibitory control of cortical inputs in the dopamine-depleted striatum that enables the augmented cortico-basal ganglia synchronization that spreads beyond the beta oscillation range.

Disclosures: V.R. Jayasinghe: None. **A.R. West:** None. **K.Y. Tseng:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

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Title: Modulating dopamine and glutamate signaling in the primary motor cortex alters the motor symptoms of Parkinson's disease and L-DOPA-induced dyskinesia in rats

Authors: *D. LINDENBACH, C. BISHOP
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Abstract: The most effective treatment for Parkinson's disease (PD) is the dopamine precursor L-DOPA, but long-term use typically results in the development of debilitating involuntary movements known as L-DOPA-induced dyskinesia. Recent research suggests that dopamine and glutamate signaling in the primary motor cortex (M1) is abnormal in PD and after L-DOPA treatment. To this end, we sought to determine if local M1 delivery of a D1 receptor antagonist (SCH23390) or an NMDA receptor antagonist (MK-801) would modify the behavioral and molecular response to a DA lesion and L-DOPA treatment. Adult male Sprague-Dawley rats were given a unilateral nigrostriatal dopamine lesion with 6-hydroxydopamine, affixed with bilateral M1 microinjection cannulae, and treated with daily L-DOPA (6 mg/kg) for 2 weeks. Subsequently, we microinjected SCH23390 (0, 3 and 18 nmol) or MK-801 (0, 3 and 18 nmol) into M1 and then delivered systemic L-DOPA (6 mg/kg). Rats were then rated on behavioral measurements of dyskinesia and PD disability. A separate cohort of rats was given the same treatments and sacrificed 1 h later for immunohistological examination of c-Fos in the motor

striatum. Initial results suggest that M1 blockade of D1 receptors reduces dyskinesia while antagonism of M1 NMDA receptors exacerbates L-DOPA-induced dyskinesia. Research linking these behavioral effects with striatal c-Fos expression is ongoing. Our data suggest that modulating aberrant M1 neurotransmitter signaling may provide therapeutic benefit for PD and dyskinesia.

Disclosures: D. Lindenbach: None. **C. Bishop:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

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Title: Optimized temporal patterns of stimulation suppress oscillatory neuronal activity in a computational model of Parkinson's disease and in hemi-parkinsonian rats

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Abstract: Non-regular temporal patterns of deep brain stimulation (DBS) are an exciting and relatively unexplored parameter space for improving therapeutic efficacy and/or efficiency. Using a computational model of the parkinsonian basal ganglia, we developed a novel temporal pattern of DBS with a low average frequency (45 Hz) for testing in hemi-parkinsonian rats and human subjects with Parkinson's disease (PD). The pattern was developed using a genetic algorithm—an engineering optimization approach—that incentivized simultaneously maximization of pattern efficacy and efficiency. Our previous work showed that the optimized pattern of stimulation suppressed PD motor symptoms in hemi-parkinsonian rats and human subjects with PD more effectively than frequency-matched regular DBS at 45 Hz. Here, we explored possible mechanisms for the differential efficacy between the optimized pattern and regular 45 Hz control DBS. We found that the optimized pattern more effectively suppressed synchronous oscillation in both a computational model of PD and in hemi-parkinsonian rats. In the computational model, the optimized pattern of stimulation suppressed oscillations in the beta range more effectively than did regular 45 Hz stimulation. In the hemi-parkinsonian rats, field potential recordings were collected from the globus pallidus and motor cortex ipsilateral to

dopaminergic lesion in awake, freely behaving rats under the different subthalamic DBS conditions. The optimized pattern of stimulation and high frequency regular DBS both significantly reduced low frequency oscillations compared to regular 45 Hz stimulation. Abnormal synchronous oscillations are thought to be related to parkinsonian symptoms, and temporal-pattern dependent suppression provides a possible mechanism of action for effective temporal patterns of DBS.

Disclosures: D.T. Brocker: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventors on licensed patents. Equity position in 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); Inventors on licensed patents. Equity position in Deep Brain Innovations, LLC..

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 414.15/S10

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS40894

Title: Relationship between oscillatory activity and tremor in rats

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Abstract: Abnormal oscillatory activity within and across cortical - basal ganglia - thalamic network is associated with motor symptoms in movement disorders (e.g. beta oscillations in Parkinson’s and theta oscillations in Essential Tremor). While pharmacological or deep brain stimulation treatment that alleviates motor symptoms may suppress these abnormal oscillations, whether such oscillatory activity is causal of motor deficits such as tremor remains unclear. Our goal was to characterize the role of abnormal oscillatory activity in motor deficits in awake and behaving healthy rats. We first focused on the so-called high voltage spindle (HVS) oscillations (5-13 Hz) that are present in the cortex – basal ganglia network in normal rats and are exaggerated with dopamine depletion. We recorded local field potentials in the motor cortex and the globus pallidus along with behavioral recordings through an accelerometer connected to the

head cap and video recordings. We observed a significant increase in spectral power and coherence between motor structures and acceleration in 7-12 Hz band during a large number of HVS episodes implying a relationship between oscillatory activity and tremulous head motion. Next, we used a computational model of the basal ganglia and a genetic algorithm optimization technique to design a novel temporal pattern of subthalamic nucleus stimulation that increased oscillatory power in the 7-12 Hz band in the basal ganglia. We then tested this pattern in normal rats and compared its effects with regular 50 Hz (the average frequency of the designed pattern), 4.5 Hz, and 9 Hz stimulation to test the hypothesis that novel stimulation pattern that increased power in the 7-12 Hz band would induce tremor in healthy rats. The optimized pattern of stimulation induced significant head and paw tremor in majority of the rats tested and severity was related to stimulation amplitude. While regular 50 Hz stimulation also induced minor motor deficits, the total number of tremulous paw movements and head tremor severity were often exacerbated by our novel stimulation pattern. While increasing stimulation amplitude of the optimized stimulation pattern exacerbated motor deficits, 4.5 or 9 Hz stimulation did not induce motor deficits even at higher amplitudes. These results suggest that when a healthy motor network is subjected to abnormal oscillatory activity through a novel stimulation pattern, the imposed activity manifests as tremor. Additionally, our results reinforce that the temporal pattern of stimulation is an important parameter that can induce selective motor deficits in a healthy animal.

Disclosures: **C.S. Oza:** None. **D.T. Brocker:** None. **C.E. Behrend:** None. **W.M. Grill:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS040894

NIH T32 NS051156

NIH R21 NS085539

Title: Neuronal basis of deep brain stimulation of the substantia nigra pars reticulata to treat gait

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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus improves the distal motor symptoms of Parkinson's disease (PD), but long-term improvements in gait and postural instability are inconsistent. Due to the substantial impact of gait and postural instability on quality of life, alternative targets for DBS are under investigation, including the substantia nigra pars reticulata (SNr). The effects of stimulation location within the large nuclear region of the SNr and stimulation parameters on improvement in gait are unclear, and this lack of foundational knowledge hinders the application and optimization of SNr DBS. We hypothesized that the location of stimulation within the SNr (medial *vs.* lateral) differentially treats gait and investigated the neuronal basis for these differential effects by characterizing the neuronal response to SNr DBS using immunomapping of markers for neuronal activity and plasticity. Rats were implanted with platinum iridium stimulating electrodes in either the medial or lateral SNr and a cannula in the medial forebrain bundle for administration of 6-hydroxydopamine to render rats hemiparkinsonian. Gait was quantified using a motorized treadmill. Immunohistochemistry was used to determine the extent of the 6-hydroxydopamine lesion, the location of the electrodes, and recent neuronal activity and plasticity as indicated by expression of c-Fos and phosphorylated extracellular signal-regulated kinase (pERK). Hemiparkinsonian rats, in contrast to healthy rats, were unable to maintain walking speed on a motorized treadmill and spent most of the time at the back of the treadmill. High frequency DBS of the medial SNr, but not lateral SNr, improved the rat's ability to maintain walking speed on the treadmill. Immunomapping showed that stimulation of the medial SNr, but not lateral SNr or non-stimulated controls, induced expression of cFos and pERK in the midbrain locomotor region (MLR), comprised of the cuneiform nucleus and pedunculopontine nucleus. These results suggest that improvement in gait depends on the location of the electrodes (medial *vs.* lateral SNr). Further, immunomapping results suggest that medial SNr DBS improves gait through modulating MLR activity and thereby shed light on the neuronal basis of SNr DBS for the treatment of PD.

Disclosures: G.C. McConnell: None. **W.M. Grill:** None.

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

Support: Columbia Udall Center

The JPB Foundation

Parkinson's disease foundation

Swedish research council

Sweden america foundation

Title: Dysfunctional GABAB transmission at striatonigral synapses drives motor sensitization in parkinsonian mice

Authors: *A. BORGKVIST[1], M. KHEIRBEK[2], R. HEN[3], D. SULZER[1]
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Abstract: Degeneration of dopamine (DA) neurons in Parkinson's disease (PD) causes hypokinesia, and subsequent DA re-administration produces highly sensitized motor behaviors in PD models. This feature is classically attributed to adaptive responses in DA receptor signaling within striatal medium spiny neurons (MSN). However, the sensitization may, in addition, reflect changes in MSN excitability due to prolonged absence of DA. Indeed, here we show that optogenetic activation of striatonigral afferents within the substantia nigra reticulata (SNr) of hemiparkinsonian mice directly produces a sensitized motor response. With patch-clamp electrophysiology and live 2-photon imaging of vesicular fusion we found increased striatonigral synaptic activity on the lesioned side, which resulted from the loss of normal GABAB mediated presynaptic inhibition. Thus, heterosynaptic sensitization of GABA release within the SNr of the parkinsonian brain is a DA receptor independent mechanism, which we propose may contribute to the sensitized motor response observed upon DA re-administration in PD.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: R01 NS079623

Ed Rudman Foundation

RJG Foundation

Title: Chemogenetic inhibition of the subthalamic region of parkinsonian mice improves motor function

Authors: *L. BROOM[1], T. SAMARDZIC[1], A. WORLEY[1], J. CLARK[1], Y. OISHI[2], D. K. SIMON[3], C. B. SAPER[3], V. VANDERHORST[3]
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Abstract: Deep brain stimulation is an effective treatment of motor symptoms in Parkinson's disease (PD). However, the presence of hardware and unintended stimulation of fiber tracts are limitations of this technology. Chemogenetic approaches provide an alternative without these limitations. One such approach makes use of Ivermectin (IVM) channels, which are mutated nematode glutamate gated chloride channels that can be delivered locally via microinjection of an adeno-associated viral vector (AAV). IVM channels are inert in mammals due to a mutation, but can be specifically and reversibly targeted by the drug Ivermectin, leading to inhibition of transfected neurons. In this study, we apply this strategy to the subthalamic region (STN), which is overactive in PD, to assess the feasibility of this approach to ameliorate motor function in an acute parkinsonian mouse model. We placed micro-injections of AAV10-CMV-IVM-GFP (Patrick Fuller) into the STN region of male C57Bl6J mice. Following transfection, we assessed the effect of inhibition of the STN region on motor function in the intact CNS following i.p. injections of vehicle or Ivermectin. To study whether the treatment effects were reversible, treatments were repeated after a wash-out period. Motor tests included high speed gait and kinematic analysis, and assessment of complex motor behavior using rotarod, balance beam, horizontal ladder, open field testing, and grip strength. To determine the effects of STN inhibition in an acute parkinsonian model, we injected 6-hydroxydopamine (6OHDA) into the substantia nigra (SN), and repeated the motor tests with vehicle and Ivermectin treatments. After the behavioral tests, we used immunohistochemistry to visualize GFP+ transfected neurons in the STN region and tyrosine hydroxylase (TH) neurons in the SN. Behavioral test results were correlated with the injection sites, and the loss of TH staining. The results show that chemogenetic inhibition of the STN region in non-lesioned mice induces small enhancements of motor performance such as an increase in gait speed and latency to drop off an accelerating rotarod. Following 6OHDA-induced dopaminergic cell loss and subsequent worsening of motor performance, inhibition of the STN region results in marked improvements of motor function. For some of the motor tests, these effects surpass pre-lesion performance. The effects are reversible and dose dependent. These findings represent a proof of concept for this novel

technology to ameliorate motor function in a parkinsonian mouse model. The results demonstrate its potential as a treatment in PD and other neurological conditions that involve an imbalance in neural circuitries.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation for Parkinson's Research Target Validation Program

AASDAP

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INCT INCEMAQ – Program National Institutes of Science and Technology of CNPq/MCT

Title: Spinal cord stimulation alleviates motor symptoms and decreases beta oscillatory activity in bilateral 6-OHDA marmosets

Authors: *R. A. FUENTES[1], M. SANTANA[2], P. HALJE[3], H. SIMPLICIO[2], U. RICHTER[3], M. FREIRE[2], P. PETERSSON[3], M. NICOLELIS[4]
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Abstract: Although deep brain electrical stimulation can alleviate the motor symptoms of Parkinson's disease (PD), a small fraction of PD patients can take advantage of this procedure due to its invasive nature. A significantly less invasive method - epidural spinal cord stimulation (SCS) - has been recently suggested as an alternative approach for symptomatic treatment of PD. However, the mechanisms underlying motor improvements through SCS are unknown. Here, we show that SCS reproducibly alleviates motor symptoms in a primate model of PD. Simultaneous neuronal recordings from multiple structures of the cortico-basal ganglia-thalamic loop in PD

monkeys revealed abnormal highly synchronized neuronal activity within each of these structures and excessive functional coupling among them. SCS disrupted this pathological circuit behavior in a manner that mimics the effects caused by pharmacological dopamine replacement therapy or deep brain stimulation. These results suggest that SCS should be considered as an additional treatment option for PD-patients.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 414.20/T3

Topic: C.03. Parkinson's Disease

Title: Phase amplitude coupling in Parkinson's Disease detected with scalp electroencephalography

Authors: *N. C. SWANN[1], C. DE HEMPTINNE[2], J. OSTREM[3], R. KNIGHT[4], P. STARR[2]
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Abstract: Introduction: Emerging theories of Parkinson's Disease (PD) suggest that it is characterized by excessive synchronization in the beta frequency band (~20 Hz) throughout basal ganglia-thalamo-cortical loops. Recently we showed that one robust measure of this synchronization is the coupling of beta phase to broad-band gamma amplitude, thought to reflect spiking activity (i.e. phase amplitude coupling, or PAC). Invasive electrocorticography recorded during deep brain stimulation (DBS) surgery revealed that PAC over primary motor cortex (M1) was elevated in PD compared to patients without PD. Other recent work suggests high frequency (>70 Hz) activity may be detectable at the scalp using electroencephalograhy (EEG), under some circumstances. We tested whether PAC (beta phase to broad-band gamma amplitude) over M1, recorded non-invasively with EEG, differs between medicated, unmedicated PD patients, and healthy control subjects and whether it is sensitive to therapeutic basal ganglia DBS. Methods: We examined an archival data set of 15 PD patients and 16 age-matched healthy control subjects. All EEG data were collected at rest. PD patients were tested on two different days, once on and once off medications, in a counterbalanced order between patients. We also collected preliminary

data from 2 PD patients with DBS. These patients were tested on and off DBS in the off medication state. For each dataset we calculated PAC using a Kullback–Liebler based method after standard pre-processing and artifact rejection. Results: For the group analyses we observed significantly greater PAC for the patients off compared to on medication ($p<0.0067$, using a two-tailed, paired, non-parametric Wilcoxon sign rank test.) The difference between patients off medication and healthy controls trended towards significance ($p<0.0604$, two-tailed, unpaired, non-parametric Wilcoxon rank sum test). The difference between patients on medication and healthy controls was not significant. DBS stimulation was associated with a suppression of PAC that rebounded once DBS is turned off. Conclusion: These preliminary findings suggest that PAC is a neurophysiological biomarker for PD in the off-medication state, and is detectable at the scalp. Future studies will investigate the reliability of the signal over-time and its relation to specific symptoms.

Disclosures: **N.C. Swann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of California, San Francisco. **P. Starr:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of California, San Francisco. **R. Knight:** None. **C. De Hemptinne:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of California, San Francisco. **J. Ostrem:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of California, San Francisco.

Poster

414. Parkinson's Disease: Circuit Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson’s Disease

Support: NSC Grant NSC101-2314-B-002-182

NTUH Grant NTUH.103-S2485

Title: The resting functional connectivity change in Parkinson’s disease related pain

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Abstract: Introduction: Pain is a disturbing non-motor symptom involving more than 40% of Parkinson's disease patients. Given that central mechanism of PD related pain remains unclear, we presume the cause of rising pain to be the intrinsic cerebral network change, and resting state functional MRI is adopted to investigate cerebral functional network. Method and result: Three groups of subjects are collected, including 12 healthy controls (HC group), 12 PD patients with pain (whose average painful VAS score >40/100) (PD-P group), and 10 PD patients without pain (PD-NP group). Demographically, the PD-P and the PD-NP groups share similar disease duration, age and sex, while the PD-P group has significantly higher UPDRS motor score, higher depression and anxiety scores. We performed seed-based analysis using substantia nigra (SN) and lentiform nucleus (LN) as seeds. Comparing to the PD-NP group, the PD-P group demonstrates significantly higher resting functional connectivity (rFC) between left LN and bilateral insula, lower rFC between right LN and left striatum, and also between left LN and right striatum. Interestingly, comparing to PD-P subjects and HC group, the PD-NP subjects have the highest rFC between LN and the opposite site striatum. In those PD subjects with initial motor symptoms onset on the left side, the PD-P subjects have higher rFC between right SN and right middle cingulate cortex (MCC). After taking UPDRS motor score, depression and anxiety scores into analysis of covariance, the rFC between right SN and right MCC is significantly higher in PD-P group, and is also positively correlated to the VAS pain scale. Discussion: We found that PD related pain closely correlates to anxiety and depression, indicating that the affective component of pain processing that is relevant to PD pain. Further evidence is provided by the resting state functional connectivity study, which shows the relationship between PD related pain and the increased rFC between SN and limbic system. Also, the increment of rFC between bilateral basal ganglia in PD-NP subjects shows a compensatory mechanism which protects the PD-NP group from having pain, and further suggests a modulatory role of basal ganglia in pain processing.

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Poster

414. Parkinson's Disease: Circuit Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH F32 NS083240-01

Title: Acute effect of lead insertion into the basal ganglia on primary motor cortex in patients with Parkinson's disease

Authors: *N. C. ROWLAND[1], C. DE HEMPTINNE[2], N. SWANN[2], R. KNIGHT[3], P. STARR[2]
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Abstract: Objectives An emerging hypothesis regarding akinesia and rigidity in Parkinson's disease (PD) posits that abnormal coupling of low-frequency beta (13-30 Hz) phase and broadband gamma (BBG) amplitude (70-200 Hz) within the cortico-basal ganglia (BG) loop prevents normal processing of motor control signals. Following surgical insertion of BG deep brain stimulation (DBS) electrodes in awake patients, there is often a temporary reduction in motor symptoms due to acute disruption of BG activity, termed the "microlesion effect". This effect should be associated with changes in motor cortex physiology. **Methods** We analyzed high-density electrocorticographic (ECOG) activity from 28-contact subdural strips placed directly over premotor, primary motor and primary sensory cortices during surgery for DBS lead placement in 10 patients with PD. Patients performed reach-and-point movements to a touchscreen tablet during a cyclical 'rest'-'prepare'-'move' sequence timed with visual cues. We compared our findings to five patients with essential tremor (ET) also undergoing DBS surgery. **Results** We found that the coupling of beta phase to BBG amplitude during rest was significantly higher in PD patients compared to patients with ET. This phase-amplitude coupling (PAC) was highest in primary motor cortex (PMC) in both groups. In PD patients, following placement of the DBS lead, PAC acutely decreased in PMC while movement-related BBG spectral power increased over both primary motor and sensory cortices. Five out of ten PD patients experienced improvement in arm rigidity within 1-3 min of lead insertion. **Conclusions** We have shown that PAC in patients with PD exceeds that of a comparison group without basal ganglia dysfunction during the resting state. In PD patients, this elevated coupling decreases acutely following DBS lead placement, a change restricted to primary motor cortex. During a whole arm reach task in PD patients, the degree of movement-related BBG synchronization increases over a distributed area of the sensorimotor cortex following DBS lead placement. We conclude that the so-called 'microlesion effect' in PD, in which temporary improvement in symptoms is seen acutely in some patients after DBS lead placement and prior to electrical stimulation, may be mediated in part by decreased phase-specific entrainment of cortical BBG activity by beta oscillations, resulting in a more robust gamma signal able to resume efficient processing of motor control signals.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.01/T6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ekam Imaging

Title: Studies on a transgenic (zQ175) model of Huntington's disease using functional imaging in awake mice: evidence of huntingtin-associated-protein 1 dysfunction

Authors: *J. R. YEE[1], P. KULKARNI[1], W. KENKEL[1], S. TODDES[2], M. NEDELMAN[3], C. F. FERRIS[1]
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Abstract: BOLD imaging in awake mice was used to identify differences in brain activity between wild-type, HETzQ175 and HOMzQ175 genotypes in response to the odor of almond. The study was designed to see how alterations in the huntingtin gene in a mouse model of Huntington's disease would affect the perception and processing of almond odor, an evolutionarily conserved stimulus with high emotional and motivational valence. Moreover, the mice in this study were "odor naive" i.e. never having smelled almond or any nuts. Using a segmented, annotated MRI atlas of the mouse and computational analysis, seventeen out of one-hundred and sixteen brain regions were identified as responding differently to almond odor across genotypes. These regions included the glomerulus of the olfactory bulb, forebrain cortex, anterior cingulate, subiculum and dentate gyrus of the hippocampus, and several areas of the hypothalamus. In many cases these regions showed a gene-dose effect with HETzQ175 mice showing a reduction in brain activity from wild-type that is further reduced in HOMzQ175 mice. Conspicuously absent were any differences in brain activity in the caudate/putamen, thalamus, CA3 and CA1 of the hippocampus and much of the cortex. These targeted areas in Huntington's disease, that show dramatic changes in function and morphology with disease progression, were not identified with the presentation of almond odor. Instead, the pattern of brain activation differentiating these genotypes fit very closely with the reported neuronal localization and concentration of huntingtin-associated-protein 1. The imaging findings suggest that the mutant Htt protein expressed by the HETzQ175 and HOMzQ175 genotypes interferes with one of the reported functions of huntingtin-associated-protein 1 - regulation of feeding behavior.

Disclosures: **J.R. Yee:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research, Ekam Imaging. **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. **M. Nedelman:** 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.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.02/T7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation, Inc.

Title: Lack of benefits from a moderate decrease of full length huntingtin by antisense oligonucleotides in a knock-in mouse model of Huntington's disease

Authors: N. R. FRANICH[1], M. A. HICKEY[1], N. H. BOVE[1], A. RELANO-GINES[1], V. LEMESRE[1], C. ZHU[1], E. R. S. TORRES[1], J. W. CHEUNG[1], S. D. S. GREWAL[1], S. LEE[1], S. KAWAKATSU[1], K. FERGUSON[1], J. USUI[1], G. DUTTA[1], W. REINDL[2], F. HERRMANN[2], D. MACDONALD[3], E. V. WANCEWICZ[4], G. HUNG[4], C. MAZUR[4], F. BENNETT[4], *M.-F. CHESSELET[1]
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Abstract: We have previously shown that short term (2 weeks) administration of antisense oligonucleotides (ASO) dose-dependently decrease the levels of huntingtin in the brain of Q140 mice, a knock-in model of Huntington's disease (HD) that presents robust behavioral, pathological and molecular deficits from a young age. Specifically, Htt ASO 75ug/d decreased Htt mRNA and HTT protein ~50% immediately following termination of treatment and the effect persisted several weeks. In the present study we tested the efficacy of the same Htt ASO that targets both mutant and wild type (WT) huntingtin mRNA to ameliorate deficits in heterozygous Q140 knock-in (Het Q140) mice. Lead anti-Htt ASO (30 or 75ug/d), a control,

RNase H-inactive, ASO (30 or 75ug/d), or sterile saline vehicle were infused intracerebroventricularly (ICV) in male Het Q140 mice or WT littermates at 5.5 months. Mice were tested at 7 months in Open Field (4 weeks post-treatment), at 7.5 months in Holeboard cognitive test (6 weeks post-treatment) and at 8-8.5 months for running wheel activity (8-10 weeks post-treatment). Behavioral deficits in Het Q140 mice were not ameliorated by lead Htt ASO at either dose. Striatal transcripts for enkephalin, dopamine receptor 2, phosphodiesterase 10a, cannabinoid receptor 1 and DARPP-32 were decreased in 8.5 months Het CAG140 mice, with no effect of ASO treatment. Total Htt mRNA and WT and mutant Htt protein levels were still decreased ~35% in striatum, cortex and posterior brain regions at ten weeks post-treatment in 8.5 months old Het Q140 mice administered with lead Htt ASO 75ug/d. In contrast, as expected, Htt ASO did not significantly affect the levels of an aberrantly spliced exon 1 Htt mRNA that encodes an N-terminal mutant HTT fragment and was detected in Het Q140 mice striatum. The results indicate a lack of beneficial effects of lead Htt ASO in Het Q140 mice despite sustained reduction of 35-50% of full length Htt mRNA and HTT protein for up to 3 months.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.03/T8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Institute of Health Grant NS41574 (MSL, CC)

Department of Veterans Affairs Merit Review Program (CKM)

Title: Alterations in thalamostriatal glutamate input to the direct and indirect pathway striatal medium spiny neurons in the R6/2 mouse model of Huntington’s disease

Authors: *C. K. MESHUL[1,2], C. MOORE[1], C. CEPEDA[3], M. S. LEVINE[3]
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Abstract: Huntington's disease (HD) is a progressive neurological disorder with symptoms of chorea and cognitive disturbances. HD is characterized by the loss of neurons primarily within the striatum and cerebral cortex. The R6/2 is a rapid progressing model of HD and can be used to determine synaptic changes by 90 days of age. Alterations in corticostriatal function and glutamate release have been well characterized in this model (Raymond et al., 2011). Using the Q140 HD animal model, Deng et al. (2013) reported a long-term decrease in the number of striatal nerve terminals making an asymmetrical synaptic contact originating primarily from the thalamus with no change in the number of contacts projecting from the cortex. We determined the changes in the relative density of nerve terminal glutamate immuno-gold labeling in identified terminals originating from either the motor cortex (vesicular glutamate transporter 1:VGLUT1) or thalamus (vesicular glutamate transporter 2: VGLUT2) that were contacting either the direct pathway (dopamine D1 receptors) or the indirect pathway (dopamine D2 receptors) medium spiny neurons. We find that within the dorsolateral striatum, there was a 38% decrease in the number of VGLUT2 (thalamostriatal) positive synaptic contacts onto spines in the R 6/2 vs the wildtype group (WT) per field of view (14 μm2) ($p \leq 0.0003$). There were no changes in the number of VGLUT1 (corticostriatal) contacts. The density of glutamate immuno-gold labeling within nerve terminals contacting direct pathway spines was significantly decreased in R6/2 mice (WT: 113.9±2.7 particles/μm2 vs R6/2: 92.4±4.4, $p \leq 0.001$) but increased in terminals contacting indirect pathway spines in the R6/2 mice vs WT (WT: 95.2±3.6 vs R6/2: 127.5±4.9, $p \leq 0.001$). There was a decrease in the density of glutamate immuno-gold labeling within the thalamostriatal positive terminals (WT: 80.3±2.1 vs R6/2: 65.2±3.2, $p \leq 0.001$) but no change in the density of glutamate labeling from corticostriatal positive terminals (WT: 73.4±2.3 vs R6/2: 78.5±3.6). There was a significant decrease in the R 6/2 mice in the density of nerve terminal glutamate immuno-gold labeling from cortical terminals making synaptic contact onto either direct or indirect pathway labeled spines (WT: 89.8±3.5 vs R6/2: 75.3±2.5, $p \leq 0.006$ for direct pathway endings; WT: 99.5±4.1, R6/2: 86.5±3.9, $p \leq 0.03$ for indirect pathway endings). These data suggest that in the R6/2, the thalamostriatal pathway is significantly affected compared to the corticostriatal pathway and that the decrease in thalamostriatal nerve terminal glutamate immuno-gold labeling occurs in terminals contacting MSNs of both the direct and indirect pathways.

Disclosures: C.K. Meshul: None. **C. Moore:** None. **C. Cepeda:** None. **M.S. Levine:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.04/T9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CONACYT, Master Fellowship 512654

Title: Study of the kinetics of the GABA current in neurons of the striatum nucleus in C57BL/6 mice treated with acid 3-nitropropionic as model of Huntington's disease

Authors: J. A. GARZÓN-VAZQUEZ[1], *J. L. FLORES-HERNANDEZ[1], E. HERNÁNDEZ-ECHEAGARAY[2]
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a mutation localized in the gene encoding the huntingtin protein. Damaging mainly subcortical structures such as the basal ganglia, of which the most affected, are the caudate nucleus and putamen, which in lower mammals are referred to as striatum. Numerous mechanisms has been proposed in the pathophysiology as excitotoxicity, lack of neurotrophic support, disruption of energy metabolism and transcriptional alterations. It is postulated that interact together, allowing partially explain the selective degeneration of striatal GABAergic neurons observed in Huntington disease. Thus, murine models have provided a stage for the investigation of the pathophysiological mechanisms associated with the development and the selectivity of the pathology of Huntington's disease. Using the animal model of the 3-nitropropionic acid 3-NP, it is a toxin that is used as animal model for Huntington disease; we studied the possible compensatory change of GABA currents. We did whole cell recordings of GABA currents in medium (MSN) and large neurons (LN) acutely dissociated from striatum of mice C57BL/6. By using the voltage clamp technique (whole cell), we analyze MSN and LN. We used concentrations from 100 nM to 1000 µM of GABA. Taking the control condition as 100% of current, the current GABA in mouse treated with 3-NP shows a biphasic effects meanwhile at 100 nM of 3-NP increased GABA currents by 62.2 %, at concentrations from 3 µM to 1 mM GABA currents were decreased with an EC50 of 6.16 µM in MSN for the control and 45.1 µM for 3 -NP. In LN we found a reduction EC50 of 83.0 µM for the control and 76.2 µM for the 3-NP-treated group. The recovery time constant tau (τ), to two successive applications of the neurotransmitter, is 12.1 ± 0.7 s (n = 3 cells) for the control group and τ = 9.0 ± 0.6 s (n = 3 cells) for treated with 3-NP. In addition, we also analyze IV of GABA current

(100μM)in order to determine the equilibrium potential of IGABA and GABA current conductance. Our results suggest that 3-NP treatment induce changes in GABA currents that can explain the differential effect founded between MSN and LN from striatum in Huntington disease.

Disclosures: J.A. Garzón-Vazquez: None. **J.L. Flores-Hernandez:** None. **E. Hernández-Echeagaray:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.05/T10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant DA026430

Title: Characterizing a novel knock-in mouse model of Huntington's Disease

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Abstract: Huntington's Disease (HD) is a devastating inherited autosomal dominant neurodegenerative disease with no known cure and few palliative treatment options available. HD is characterized by progressive deterioration of motor, cognitive and psychiatric function. At disease onset, the average survival time is 15-25 years and end-stage patients are profoundly demented and debilitated. There is need for an improved HD mouse model that thoroughly recapitulates the neuropathological and symptomatic features found in human HD to better understand the pathological process and to develop novel treatments. The HdhQ mouse model was developed by knocking-in pathogenic-sized CAG repeats into the *Hdh* gene, the mouse homolog of the Huntington's Disease gene. An allelic series of the HdhQ model has been generated with repeats from 50 to 350 CAGs in length and this mouse line represents a developing, powerful mouse genetic tool. Several of these lines recapitulate critical HD pathological processes and symptoms, however it is unclear whether this holds for repeat lengths above 300. Here we seek to characterize several pathological indices and behavioral impairments in the longest of the allelic series, the newly developed HdhQ350 model. Longitudinal studies of behavioral phenotypes and histology will uncover the age of disease onset in this mouse line.

Behavioral tests will address motor, cognitive and psychiatric-like phenotypes commonly measured in HD research. Semi-quantitative immunohistochemistry will be used to study disease progression at various time points, examining classic HD molecular pathogenesis, such as mutant Huntington protein aggregation in the nucleus and cytoplasm, loss of synaptic proteins and astrocyte activation. Our preliminary results indicate that at 20 weeks of age, there is no distinguishable difference in behavioral phenotypes between HdhQ350 mice and their wild-type littermates. Our studies are designed to uncover accelerated motor and cognitive decline, as well as mutant Huntington aggregation and reduced synaptic markers in the striatum in the HdhQ350 mouse line. These studies will disclose if the HdhQ350 mouse line is an improved model for the elucidation of pathogenic pathways in HD, providing a foundation for future HD studies and drug discovery.

Disclosures: J.K. Cao: None. **P. Detloff:** None. **N. Stella:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.06/T11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Alterations in sensory-evoked oscillatory activity in mouse models of Huntington's disease

Authors: *M. E. LEVIN[1], K. A. RICHARDSON[2], B. BURAN[2], E. D. BUERGER[2], B. K. ESCHLE[2], R. LEE[3], J. K. T. WANG[3], D. J. GERBER[2]
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Abstract: Early stages of Huntington's disease (HD) are characterized by subtle dysfunctions in cortical synaptic and network physiology that may contribute to motor and cognitive deficiencies and lead to later stage neurodegeneration. Therapeutic intervention at early stages could halt or reverse disease progression prior to gross neuronal loss. Identification of an EEG biomarker to detect early neuronal network dysfunction in HD animal models and in humans would provide a valuable tool to guide therapeutic discovery and development. In previous studies (Cottrell et al., SFN 2012, 154.02), we observed alterations in network activity in HD mouse models (zQ175, BACHD) in prefrontal cortex during habituation to a novel environment and in auditory cortex

during an auditory steady state response paradigm (ASSR, auditory click trains over a range of discrete frequencies: 20-100 Hz). We have extended these studies, performing a longitudinal study of the zQ175 model as well as additional HD models (HdhQ20, HdhQ80) using the ASSR paradigm. Alterations in the steady state response recorded in auditory cortex were observed in all HD models at all ages tested. Preliminary results indicate the potential of utility of ASSR as an EEG biomarker for HD.

Disclosures: **M.E. Levin:** A. Employment/Salary (full or part-time):; Galenea Corp. **K.A. Richardson:** A. Employment/Salary (full or part-time):; Galenea Corp. **B. Buran:** A. Employment/Salary (full or part-time):; Galenea Corp. **E.D. Buerger:** A. Employment/Salary (full or part-time):; Galenea Corp. **B.K. Eschle:** A. Employment/Salary (full or part-time):; Galenea Corp. **R. Lee:** A. Employment/Salary (full or part-time):; CHDI Foundation. **J.K.T. Wang:** A. Employment/Salary (full or part-time):; CHDI Foundation. **D.J. Gerber:** A. Employment/Salary (full or part-time):; Galenea Corp.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI grant A7335

Title: Mutant-huntingtin transgenic songbirds: A new model for the study of progressive vocal learning disorder

Authors: ***W.-C. LIU**, J. KOHN, S. SZWED
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Abstract: Progressive speech or vocal disorder characterizes many neurodegenerative diseases; however, the lack of complex vocal-learning behavior in current mammalian models makes it difficult to study deteriorating vocal-motor learning within specialized brain regions. Vocal learning in songbirds is a complex and highly quantifiable vocal-motor behavior. The learning and production of a song involves a well-defined and specialized cortico-basal ganglia (CBG)“song circuit” that is comparable to human brain regions used for speech learning. We use transgenic songbirds as a genetic model for the study of progressive vocal-learning disorder in Huntington’s Disease (HD). HD is a genetic, neurodegenerative disorder caused by mutation of

the huntingtin (Htt) gene. Subsequent mutant-huntingtin protein induces neuronal degeneration in the CBG circuit, which results in progressive motor dysfunction. Here we created transgenic songbirds expressing lentiviral-mediated human full-length mutant-huntingtin gene (145Q). Remarkably, the mutant birds show severe and progressive vocal learning disorders that are comparable to those of human HD. These vocal deficits include poor imitation of tutor song, progressive song degradation, sustained variability in syntax and syllable structures, and variation in the timing of song crystallization. Additionally, the degree of vocal disorder in mutant birds is correlated with down-regulation of dopamine and androgen receptor expression in the CBG "song circuit". These transgenics are the first experimentally created songbird functional mutants, making songbirds a powerful new animal model system to explore and manipulate the genetic mechanisms and brain circuit function that underlie complex vocal learning or vocal disorders.

Disclosures: W. Liu: None. **J. Kohn:** None. **S. Szwed:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

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Program#/Poster#: 415.08/U1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Muscular volumetric and metabolite characterization of R6/2 and zQ175 knock in mice of Huntington's disease

Authors: K. LEHTIMÄKI[1], T. LAITINEN[1], T. HEIKKINEN[1], A. NURMI[1], *O. M. KONTKANEN[1], I. MUNOZ-SANJUAN[2], L. C. PARK[2]
[1]Charles River Discovery Res. Services, Kuopio, Finland; [2]CHDI Management/CHDI Fndn., Los Angeles, CA

Abstract: HD patients suffer from debilitating and progressive motor defects such as chorea, rigidity, dystonia and general muscle weakness and wasting. In the current study, we have characterized the MR imaging (MRI) -based volumetric changes in gluteus maximus and quadriceps muscles in R6/2 (4 and 12 weeks age) and zQ175KI (6, 9 and 12 months) mice to assess HD-associated muscular changes in rodent models of HD. In addition, ^{1}H MR spectroscopic (MRS) data from quadriceps muscle were acquired to evaluate whether muscle wasting is accompanied with low-molecular weight metabolite and fatty acids changes. MRI and MRS experiments were carried out using a horizontal 11.7 Tesla Bruker BioSpec small animal

scanner. Smaller but non-significant gluteus maximus and quadriceps muscle volumes between the wild-type and R6/2 transgenic mice was detected at 4 weeks old. At 12 weeks of age, the muscle volumes in WT mice increased by over 40%; whereas, those in R6/2 didn't increase. In zQ175KI, muscles at 6 months old homozygotes and 9 months old heterozygotes display the significant volume loss compared to those in wild-type littermates and continued to have the muscle volume loss (>20%) over the next three months. R6/2 quadriceps muscle showed mild genotype differences in ^{1}H MRS analysis compared to wild-type littermates at 4 weeks old, with increase in the intra-myocellular lipids, taurine and total cholines, but no significant changes at 12 weeks of age. Based on these observations, HD related muscle wasting in rodents does not seem to be associated with significant fatty acid accumulation. These MRI/MRS readouts may help to further understand the muscle pathophysiology of HD as well as to develop a potential biomarker for the disease and therapeutic application.

Disclosures: K. Lehtimäki: None. **O.M. Kontkanen:** None. **T. Laitinen:** None. **A. Nurmi:** None. **I. Munoz-Sanjuan:** None. **L.C. Park:** None. **T. Heikkinen:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.09/U2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Early fine motor skill impairment of R6/2 and zQ175 knock in mice of huntington's disease

Authors: T. HEIKKINEN[1], T. BRAGGE[1], *J. T. PUOLIVALI[1], A. NURMI[1], O. KONTKANEN[1], I. MUNOZ-SANJUAN[2], L. C. PARK[2]
[1]Charles River Discovery Res. Services, Kuopio, Finland; [2]CHDI Management/CHDI Fndn., Los Angeles, CA

Abstract: R6/2 mice and zQ175 knock-in (KI) mice of Huntington's disease (HD) exhibit progressive gross motor deficits as studied with conventional sensory motor tests. We have previously shown characteristics of fine motor skill deficits of R6/2 and Q175 mice at symptomatic age of 10-12 weeks. In this study, we characterized fine motor deficits in these mice at early, 4-5 weeks of age using an automated high precision movement analysis system. HD and wild-type mice were tested at three modes of moving: walking, ladder walking and wading. The gait was recorded with a high speed camera, imaged and analyzed simultaneously

from three spatial dimensions, via comprehensive kinematic algorithms. Body parts were marked with a contrast agent to enhance point-wise detection in the x- and y-axis. Each point of movement trajectory was calculated as a change in coordinates and used for data analysis. The parameters analyzed, including gait characteristics and fine motor movements, were compared between the genotypes to reveal disease specific defects. Results indicate that both R6/2 and zQ175KI mice display phenotypical HD-like fine motor deficits already at an early age of 4-5 weeks, such as lowered back body postures and increased angle ranges of the limb joints. Specific deficits are observed in the more challenging modes of movement, i.e. in ladder walking and wading. This method is applicable not only to study motor defect development in rodent models of HD and other motor impairment diseases, but also to capture very early, more subtle pathological symptoms with fine motor skill changes, and may offer a sensitive tool to investigate the efficacy of therapeutic drugs that improve early and subtle motor functions.

Disclosures: T. Heikkinen: None. **J.T. Puolivali:** None. **T. Bragge:** None. **A. Nurmi:** None. **O. Kontkanen:** None. **I. Munoz-Sanjuan:** None. **L.C. Park:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: DGAPA-PAPIIT No. IN201307

CONACyT No. 81062

Title: Modulation of GABAergic plasticity in a mice model of striatal degeneration

Authors: E. NIETO-MENDOZA, *E. HERNANDEZ-ECHEAGARAY, Dr
Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstract: Dopamine (DA) is involved in several functions of basal ganglia like motor functions, cognition and synaptic plasticity. In the striatum, DA modulates synaptic transmission, as well as short and long term synaptic plasticity. Activation of D1 dopamine receptors (D1), increases glutamatergic currents of corticostriatal pathway, and stimulation of D2 dopamine receptors (D2) attenuates them. In addition D1 and D2 activation play a part in corticostriatal long-term depression (LTD), while D1 plays a role in the long-term potentiation (LTP). DA also modulates

short term depression (STD) in GABAergic synapses; however it is not well known how DA participates in long term plasticity of striatal inhibitory synapses. As local circuits in the striatum are GABAergic, and MSNs activity is under the control of GABAergic interneurons (GI), in this work we analyze both dopaminergic modulation in GABAergic synapses and synaptic plasticity between the GI and MSNs. Our results demonstrated that DA was important for synaptic plasticity. Differential activation of DA receptors with specific agonist or DA in high or low concentration modifies plasticity; high concentrations of DA (20μM) induced LTP mainly while low concentration of DA (200nM) produced LTD. Modulation of DA on GABAergic synapses and in striatal plasticity was compared in an animal model of striatal degeneration induced with the mitochondrial toxin 3-nitropropionic acid (3-NP). Striatal damage changed plasticity and DA modulation of GABAergic synapses. Interestingly DA in high concentration mimics the plasticity induced in the animal model of striatal degeneration.

Disclosures: E. Nieto-Mendoza: None. **E. Hernandez-Echeagaray:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NSO71456

Title: Accelerated disease progression in the r6/2 mouse model induced by the tetracycline transactivator

Authors: K. L. WHEATON[1], K. OBRIETAN[2], *K. R. HOYT[1]
[1]Pharmacol., [2]Neurosci., Ohio State Univ., COLUMBUS, OH

Abstract: The tetracycline-controlled transcriptional activation system is a popular, and powerful method for enhancing or mitigating expression of a gene *in vivo*. We are interested in using this system to overexpress genes of interest in the r6/2 mouse model of Huntington's disease (HD) as a means to slow the progression of disease. As our research was ongoing, Han et al 2012 (J Neurosci. 31:10574) reported deleterious hybrid strain effects when the tetracycline transactivator (tTA) protein was driven in neurons using the calcium/calmodulin-dependent kinase II α (CaMKIIα) promoter, similar to our experimental approach. Since the r6/2 mice are a hybrid strain of C57BL/6 and CBA mice, this potentially complicates the interpretation of our

results. To assess the impact of neuronal tTA expression in the hybrid background r6/2 strain, we studied four cohorts of animals, HD, WT, HD/tTA and WT/tTA. We determined the progression of HD by measuring motor coordination and weight, as these are evident markers of disease in human patients. Starting at four weeks of age and continuing weekly until euthanized at ten weeks, mice in each cohort were challenged on the rotarod and weighed. Interestingly, mice in the HD/tTA cohort performed significantly worse on the rotarod. At six weeks HD mice failed the rotarod after 230.4s (n=15), whereas HD/tTA mice failed after 160.5s (n=11). Additionally, the HD/tTA genotype had a more rapid increase in weight from four to eight weeks, followed by a more rapid weight loss from eight to ten weeks, compared to the HD cohort. Expression of tTA did not alter rotarod performance or weight in WT mice. Preliminary results indicate that neuronal tTA expression in HD mice does not lead to increased neuronal cell death (by fluorojade labeling) nor increases in neuronal expanded-repeat huntingtin expression/aggregation (by fluorescence immunohistochemistry). Additionally, tTA expression levels were comparable in HD and WT mice (by qPCR). The aforementioned research group linked a protective gene in the C57BL/6 mice, and when diluted in a hybrid strain, led to tTA neurotoxicity. While the precise mechanism of how tTA exacerbates the progression of HD has yet to be elucidated, our results raise awareness of the deleterious impact of neuronal tTA in hybrid background animals. Strain differences may create undesired effects from the transgenic system alone, compromising the credibility of the observed gene-effect.

Disclosures: K.L. Wheaton: None. **K. Obrietan:** None. **K.R. Hoyt:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

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Program#/Poster#: 415.12/U5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Electrophysiological characterization of R6/2 and zQ175 knock in mice of huntington's disease using implanted telemetry

Authors: *H. CHADCHANKAR[1], T. HEIKKINEN[1], A. NURMI[1], O. KONTKANEN[1], I. MUNOZ-SANJUAN[2], L. C. PARK[2]
[1]Charles River Discovery Res. Services Finland, Kuopio, Finland; [2]CHDI Management/CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease (HD) is a progressive neurodegenerative disease characterized primarily by the symptoms of chorea and seizures. However, chorea has not been well studied in HD preclinical models. Moreover, the sporadic nature of subclinical and overt seizures presents a significant challenge in studying these changes in mouse models. In this study, we used implanted telemetry devices equipped with biopotentials to measure changes in electroencephalogram (EEG) and electromyogram (EMG) in HD mouse models. 7-week old zQ175 knock-in (KI) homozygous mice and 6-week old R6/2 mice were implanted with a telemetric radio transmitter. Recordings were made biweekly over a period of three months in zQ175KI homozygous mice and weekly in R6/2 mice over a period of 18 weeks. EEG was recorded with biopotentials implanted in the cortex and EMG was recorded with biopotentials implanted on trapezius and gluteus maximus muscles. Subclinical seizures, as defined by lack of overt convulsions, were observed cortical EEG in R6/2 mice from the age of 8 weeks and their prevalence increased significantly with increasing age. 10-week old R6/2 mice displayed significant electrical discharge consistent with overt seizures. Similar subclinical seizures were also found in cortical EEG in 9-month old zQ175KI homozygous mice. No such activity was observed in age-matched WT mice. Preliminary analysis of EMG revealed that the R6/2 transgenic mice show rhythmic, irregular innervation of muscles at rest possibly indicating deficits in neuromuscular junction and hyperexcitability of muscles in HD mice. These results provide a novel approach to study chronic electrophysiological alterations in HD mice to study underlying abnormalities and screen potential therapeutic drugs.

Disclosures: H. Chadchankar: None. **T. Heikkinen:** None. **A. Nurmi:** None. **O. Kontkanen:** None. **I. Munoz-Sanjuan:** None. **L.C. Park:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.13/U6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Central and autonomic dysfunction induces severe abnormalities of circadian rhythm and cardiac function in R6/2 and zQ175 knock in mice of huntington’s disease

Authors: H. CHADCHANKAR[1], T. HEIKKINEN[2], A. NURMI[1], *N. E. VARTIAINEN[1], O. KONTKANEN[1], I. MUNOZ-SANJUAN[3], L. C. PARK[3]

[1]Charles River Discovery Res. Services, Kuopio, Finland; [2]Charles River Discovery Res. Services Finland, Kuopio, Finland; [3]CHDI Management/CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease (HD) is a progressive neurodegenerative disease that produces a wide variety of symptoms in addition to primary motor deficits. However, the precise nature of these symptoms and causes of mortality are poorly understood. In this study, we characterized circadian rhythm and cardiac abnormalities in two HD mouse models using implanted telemetry device. 7-month old zQ175 knock-in (KI) homozygous and 6-week old R6/2 transgenic mice were used along with their wild-type (WT) littermates. Locomotor activity and core body temperature were monitored using intraperitoneally implanted radio transmitters equipped with biopotentials. Electrocardiogram (ECG) was recorded with biopotentials implanted in lead II configuration. Recordings were made biweekly over a period of three months in zQ175KI homozygous mice and weekly in R6/2 mice over a period of 18 weeks. Both HD mouse lines displayed significant hypoactivity during the dark cycle phase compared to WT mice. However, there was no difference between their activity during the light cycle phase. Both HD mouse lines also displayed lower core body temperature than WT mice. These HD mouse lines also exhibited significantly elevated heart rate. The difference in heart rate became pronounced from the age of 7 months and 9 weeks in Q175 and R6/2 mice, respectively. The R6/2 mice also showed age-dependent alterations in PQRST waves in ECG from the age of 7-8 weeks with clear exacerbation in cardiac wave propagation by the age of 15 weeks, including cardiac arrhythmia. Detailed frequency domain analysis of ECG revealed clear deficits in parasympathetic and sympathetic innervations of cardiac function in HD mice in comparison to WT mice, reflecting autonomic dysfunction induced by HD. Consistent with earlier studies, we also observed decreased heart rate variability in HD mice further suggesting impairment in maintaining adequate cardiac output. This study highlights that HD produces significant impairment in the circadian rhythm and cardiac function in HD mouse models. The study also highlights novel therapeutic areas to target autonomic dysfunction in HD.

Disclosures: H. Chadchankar: None. **T. Heikkinen:** None. **A. Nurmi:** None. **N.E. Vartiainen:** None. **O. Kontkanen:** None. **I. Munoz-Sanjuan:** None. **L.C. Park:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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California Institute for Regenerative Medicine DR-05415

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Roberson Family

TeamKJ

Title: Engineered mesenchymal stem cells to overexpress BDNF for the treatment of Huntington's disease

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Abstract: Huntington's disease (HD) is an autosomal dominant disorder caused by an expanded CAG trinucleotide repeat that causes a progressive degeneration of neurons in the putamen and caudate nucleus. Survival and function of striatal neurons is dependent on brain-derived neurotrophic factor (BDNF), and levels of this trophic factor are significantly reduced in HD patients. Recently, strategies aimed at BNDF restoration have become a leading candidate for the treatment of HD. Transplantation of adult stem cells, such as bone marrow-derived mesenchymal stem/stromal cells (MSC), show considerable therapeutic promise through stimulation of endogenous neuronal growth, decreased neuronal apoptosis, regulation of inflammation, and the secretion of trophic factors. MSC are readily available, easily expanded *in vitro*, have immunomodulatory properties, and can be easily engineered to over-produce trophic factors. Previously, in mouse and rat models of HD, it has been shown that allogeneic transplantations of MSC can significantly delay the onset of behavioral abnormalities and reduce the severity of neuropathological changes in both transgenic and toxic lesion models of HD. In a pivotal proof-of-concept study, transgenic mice showed significant behavioral and neuropathological sparing following the intrastriatal administration of MSC engineered to over-express BDNF. The aim of the current study is to test the pre-clinical safety and efficacy of human MSC, genetically engineered to produce BDNF, for the treatment of HD. Our developmental candidate is allogeneic human MSC engineered, using a lentiviral vector, to secrete BDNF (MSC/BDNF). Our product combines the beneficial effects of MSC administration with the benefits of sustained BDNF production. These proof-of-concept and safety studies are being conducted in support of HD-Cell, a planned future Phase I clinical trial designed to examine the safety and potential efficacy of MSC/BDNF. The current studies focus on the optimal transduction coefficients, the level of BDNF produced by our developmental candidate, the karyotypic stability of the candidate, the safety profile following transplantation in immune deficient mice, cell retention *in vivo*, and efficacy following transplantation in the YAC128 and R6/2 (120 CAG) transgenic

mouse models of HD, as IND-enabling studies for the FDA, in support of a future planned Phase I clinical trial. *Support for this project was provided by CIRM grants no. TR1-01257 (Nolta) and DR2-05415 (Wheelock/Nolta), NIH Director's transformative award 1R01GM099688 (Nolta), and philanthropic donors from the HD community, including the Roberson family and TeamKJ.*

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.15/U8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Medical Research Council

CHDI Foundation

Title: A detailed description of the Huntington's disease-like pathology exhibited by the HdhQ150/150 mouse

Authors: *I. RATTRAY[1], E. J. SMITH[1], W. R. CRUM[2], T. A. WALKER[1], R. GALE[1], G. BATES[1], M. MODO[3]
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Abstract: The HdhQ150/Q150 knock-in mouse model of Huntington's disease (HD) carries an expanded polyglutamine sequence in the mouse huntingtin homologue, resulting in the eventual development of HD-like pathology at a slower rate than the more commonly used transgenic models. Using a combination of behavioral assessments, magnetic resonance imaging (MRI) and histology, this study will provide a longitudinal, integrated analysis of the progressive pathology exhibited by this mouse line with a particular focus on the early stages of disease progression. Both male and female, wild type and HdhQ150/Q150 mice were used. To probe functional abnormalities, a detailed battery of behavioral measures was applied including rotarod, grip strength, open field, olfactory discrimination, social interaction, cued and contextual fear conditioning, as well as learning in a swimming T-maze. These tests were coupled with

longitudinal *in vivo* MRI to monitor regional brain atrophy and T2 relaxivity (reflective of brain tissue composition) at 2, 4, 6, 9, 12, 18 and 22 months of age, whereupon the mice were culled. Preliminary analyses indicate age-related loss of volume in several key brain regions. Indeed, high resolution *ex vivo* MRI was conducted prior to post-mortem histological analysis of neuropathology to further support *in vivo* measures. Tensor Based Morphometry analyses of these images revealed a substantial, widespread brain volume loss at this late age. Correlative analyses will ultimately probe links between behavioral changes and brain abnormalities determined through MRI and histology. This continuing and developing description of the HdhQ150/Q150 knock-in mouse model will provide crucial information as to the progression of HD-like pathology, as well as to what extent functional deficits are associated with neuropathological changes in this line.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Reducing caspase 6 activity by AAV-mediated RNAi partially ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease

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Abstract: Huntington’s disease (HD) is a fatal, dominant neurogenetic disorder. The molecular basis of HD has been determined to be due to an expansion of the trinucleotide CAG in exon 1 of the huntingtin gene (HTT). The resultant expansion of a polyglutamine tract in the mutant huntingtin (HTT) protein confers a toxic gain of function. Fragments resulting from proteolysis of the mutant HTT protein (mHTT) by caspases have been implicated in the pathogenesis of HD. In particular, proteolysis of mHTT by caspase-6 cleavage has been implicated in mediating neuronal dysfunction and neurodegeneration. Inhibiting caspase 6-mediated proteolysis of mHTT in the YAC128 mouse model of HD reportedly rescues all of the HD-like phenotypes

evaluated in this model. This observation of abatement of the HD phenotype in caspase-6-resistant (C6R) YAC mice (harboring 133CAG repeats in the HTT gene) suggests that caspase 6-mediated cleavage at residue 586 of HTT contributes to the development of disease. Hence, facilitating a reduction in caspase 6 activity may be protective for HD. To further validate caspase 6 as a therapeutic target for HD, we generated a recombinant AAV2/1 vector encoding a miRNA-based hairpin designed to lower expression of caspase 6 (AAV-miRNA-C6). Vectors were delivered via direct intra-striatal injections into 3 months old YAC128 mice or wild type littermate controls. Both motor and affective behaviors were evaluated and tissues were harvested for histological and biochemical analysis at 4 months post-injection. Biochemical analysis of striatal extracts from AAV-miRNA-C6-treated animals showed significantly lower levels of caspase 6 compared to AAV-null injected controls. Behavioral analysis of YAC128 mice injected with AAV-miRNA-C6 showed improvements in the rota-rod motor test for coordination and a trend towards improvement on the tail suspension test for depression. These findings support an important role for caspase 6 in HD pathogenesis and provide a rationale for developing therapeutic strategies to inhibit caspase 6 activity.

Disclosures: L.M. Stanek: None. **B. Mastis:** None. **S.P. Sardi:** None. **B. Wong:** None. **S. Ladha:** None. **D. Ehrnhoefer:** None. **M. Hayden:** None. **S.H. Cheng:** None. **L.S. Shihabuddin:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Title: Earlier deficits are associated with increased aberrantly spliced mutant huntingtin in knock-in mouse models of Huntington's disease

Authors: *N. R. FRANICH[1], M. A. HICKEY[1], A. NEUEDER[2], T. CHU[1], C. ZHU[1], N. H. BOVE[1], V. LEMESRE[1], R. P. LERNER[1], J. S. STEFFAN[3], S. O. ZEITLIN[4], G. P. BATES[2], M.-F. CHESSELET[1]
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Abstract: We have previously shown marked differences in onset/progression in fully backcrossed Q140 knock-in (KI) and HdhQ150 KI mice. These lines differ in the sequence of exon 1, with the Q140 mice carrying a chimeric human/mouse exon 1 containing an expanded CAG repeat and the human proline-rich region (PRR) inserted into the mouse Htt gene whereas the HdhQ150 KI mice have an expanded CAG repeat inserted into the Htt gene with a murine PRR. Both models exhibit ~40% neuronal loss in the striatum by 2 years of age, preceded by progressive neuropathological abnormalities and motor deficits, but Q140 mice present abnormalities and deficits at a much younger age than HdhQ150 KI mice. Specifically, backcrossed Q140 mice show decreased locomotion in the open field at 1, 4 and 6 months, impairment in the pole task at 4 months and decreased running wheel performance at 6 months. Q140 mice also show early cognitive impairment with decreased spontaneous alternation in the Y-maze at 3 months. In contrast, HdhQ150 mice were not impaired in these tests at the same ages. Furthermore, a marked difference in huntingtin (HTT) immunostaining between these mice was evident by 4.5 months of age, with Q140 mice, but not HdhQ150 mice, showing diffuse nuclear staining, microaggregates, nuclear inclusions and neuropil aggregates in the striatum and cortex using pEM48, MW8, and S830 antibodies. Both lines show similar HTT staining using 4H7H7 expanded polyglutamine antibody following antigen retrieval. Western blotting of striatal nuclear protein extracts using anti-HTT PW0595 shows that Q140 mice have 2-fold more insoluble aggregates than HdhQ150 at 6 months, whereas HdhQ150 mice show 5-fold more soluble HTT fragments (Franich et al., 2011 SfN abstract). We now show that, at 6 months, both lines have similar decreases in striatal transcripts including enkephalin, DARPP32, dopamine receptor 2 and cannabinoid receptor 1, indicating that alterations in these transcripts precede pathological and behavioral deficits in HdhQ150 mice. Notably however, the level of an aberrantly spliced exon 1 Htt mRNA that encodes a toxic N-terminal mutant HTT fragment (Sathasivam et al., PNAS 2013) is significantly higher in Q140 mice than HdhQ150 mice at 2 months of age. The results indicate that earlier deficits are associated with increased level of this aberrantly spliced Htt transcript, suggesting it may be a potentially important target for therapeutic intervention.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.18/U11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Phenotypic characterization of BACHD hemi rats of huntington's disease

Authors: J. PUOLIVÄLI[1], *S. KIM[1], T. HEIKKINEN[1], T. BRAGGE[1], K. LEHTIMÄKI[1], T. LAITINEN[1], O. KONTKANEN[1], H. NGUYEN[2], I. MUNOZ-SANJUAN[3], D. HOWLAND[3], L. C. PARK[3]
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Abstract: Huntington's disease (HD) is an autosomal neurodegenerative disorder, characterized by severe behavioral, cognitive, and motor deficits. BACHD transgenic rats (line TG5) are shown to display a robust, early onset and progressive HD-like phenotype including motor deficits and anxiety-related symptoms (Yu-Taeger et al. 2012). The purpose of this study was to further characterize the model with additional behavioral and imaging measurements. Both female and male hemi BACHD and WT rats at age of 2-13 months of age were used. Rats were tested for motor balance and coordination in rotarod, for locomotor activity in open field, for anxiety in Elevated Plus Maze, and for fine motor movements and gait properties using an automated high precision movement analysis system. Moreover, brain volumetry and metabolite levels were analyzed using MRI & 1H-MRS measurements. In a separate cohort, the BACHD and WT rats were tested in a water maze at 9-10 months of age using two different water temperatures (22°C and 28°C). BACHD rats did not differ from WT rats in their survival or general condition during the follow-up. Body weight was significantly increased in all study groups during ageing. The body weight of female BACHD rats was significantly higher compared to WT rats. Rotarod fall latency and open field activity were significantly decreased in BACHD starting as early as 2-3 months of age. In Elevated Plus Maze, BACHD rats spent more time in open arms at 6 and 10 month time points studied. No differences were observed between BACHD and WT rats in fine motor movements or gait properties. Volumetric MRI measurements revealed that BACHD rats had decreased brain volumetry starting as early as 2-3 months of age. In addition, several hippocampal and striatal metabolite changes were observed by 1-H MRS measurements at 2-10 months of age. In the water maze test, BACHD rats showed impaired reference memory acquisition compared to WT rats independent of water temperature as shown by increased distance and latency to find the hidden platform. In addition, BACHD rats

showed decreased swim speed and increased floating behavior compared to WT rats. These results confirm previously published observations that BACHD rats show clear motor deficits and anxiety-related symptoms compared to WT rats. Moreover, the BACHD rats also manifest decreased brain volumetry compared to WT rats. In conclusion, BACHD rats show several motor, emotional, cognitive and pathological changes resembling those observed in HD patients making them a valuable model for preclinical studies.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

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Program#/Poster#: 415.19/U12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI

Title: Neurophysiological biomarkers for evaluating a Phosphodiesterase9A inhibitor for Huntington’s disease

Authors: D. NAGY, F. D. TINGLEY III, M. STOILJKOVIC, *M. HAJOS
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Abstract: Several neurophysiological abnormalities have been described in Huntington's disease, including auditory gating deficit, which are considered to reflect impaired brain information-processing. Using clinically equivalent acoustic-stimulation paradigms, we have recently reported impaired auditory gating in transgenic BACHD (line5) rats under anesthesia and in freely-moving condition. The main goal of our current studies was to establish if auditory gating deficit in these transgenic animals could serve as a translational biomarker in drug development of Huntington’s disease. Phosphodiesterase (PDE) inhibitors have been considered as potential treatments for Huntington’s disease since previous findings have indicated dysregulations of PDEs activities, leading to a decrease in cyclic guanosine monophosphate levels in the striatum and hippocampus in Huntington’s disease patients and Huntington’s disease transgenic animals. Therefore, effects of PF-04447943 ((6-((3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl)-1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazolo[3,4-

d]pyrimidin-4(5H)-one), a recently developed selective phosphodiesterase9A (PDE9A) inhibitor (Kleiman et al., JPET, 341:396-409, 2012) were tested on auditory gating in BACHD transgenic rats. Electrophysiological recordings from the hippocampus CA3 region and primary auditory cortex were carried out under chloral hydrate anesthesia and non-anesthetized, freely-moving condition. PF-04447943 (1 mg/kg, sc) significantly improved auditory gating in anesthetized transgenic rats, fully abolishing the gating difference between transgenic and wild type rats. In freely moving transgenic rats, PF-04447943 dose-dependently improved their gating in both the hippocampus and primary auditory cortex. Furthermore, treatment of transgenic BACHD rats with daily administration of PF-04447943 (1 mg/kg) over 7-days resulted in an improvement in their auditory gating in both regions as evaluated 24 hours after the last treatment. In fact, differences in auditory gating and gamma band power between wild-type and transgenic BACHD rats were totally abolished after sub-chronic treatment with the PDE9A inhibitor. Our findings indicate that BACHD transgenic rats show abnormal auditory gating with features resembling those of Huntington's disease patients, which could be considered as potential translational biomarker for drug development in treatment of this disease.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 415.20/U13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Evaluation of prophylactic treatment with the phosphodiesterase 10 inhibitor MP10 on cognitive and behavioral assessments in the Q175 knock-in mouse model of Huntington's disease

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Abstract: Huntington's disease (HD), a dominantly inherited neurodegenerative disorder with no effective therapy, is caused by expansion of the polyglutamine repeat in the huntingtin (Htt) gene. The most pronounced neuropathology in HD occurs in striatal medium spiny projection neurons (MSNs) leading to dysfunction of the cortico-striatal-thalamo-cortical network.

Impairment in this circuitry is also present in HD transgenic mice bearing mutated Htt including R6/2 and Q175 mouse models. Previous reports have shown that the hyperexcitable electrophysiological phenotype that develops in cortico-striatal brain slices with disease progression can be reversed acutely by exposure to several subtype-selective phosphodiesterase inhibitors (PDEi) *in vitro* (Kleiman et al., 2011 SFN) and that chronic, 6 month administration of the PDEi MP10 improved MSN function in slices prepared from Q175 mice. The aim of the present set of experiments was to evaluate behavioral and cognitive effects of long term, prophylactic treatment of MP10 in Q175 mice beginning in early adolescence, prior to the onset of disease progression. Mice began daily chronic treatment of MP10 at 2 months of age and were assessed for acquisition of touchscreen visual discrimination and reversal learning tasks in separate cohorts: cohort A) early training (acquisition began in pre-diseased mice at 4 months) and cohort B) training began at 6 months of age at a timepoint when disease symptoms are observed. The results of these studies and additional behavioral measures including motor assessments (open field and inverted screen tests), and activities of daily living (nesting) will be presented.

Disclosures: **P. Stolyar:** A. Employment/Salary (full or part-time):; Pfizer Inc. **C. Arturi:** None. **D. Volfson:** None. **S. Lotarski:** None. **S.J. Sukoff Rizzo:** None. **M.M. Zaleska:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NeuroModel (FP7 ITN)

Title: Application of home cage monitoring in the behavioural characterization of Huntington R6/2 transgenic mouse model: An early diagnostic tool?

Authors: *R. DE HEER[1], M. MELLACE[2], G. BATES[3], B. M. SPRUIJT[2]
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Abstract: A lot of CNS compounds developed by pharmaceutical companies failed to get to the clinic and there is a need for more valid and faster animal models with more translational value. Many conventional tests have certain limitations and automated home cage monitoring could be

a solution. Not only is the latter as free from human intervention as possible, it provides a rich and varied environment and, thus, maximizes ethological validity, animal welfare and relevant behavioral challenges during (automated) behavioral tests. Because computer software takes care of monitoring, animals can be observed 24 hours a day, 7 days a week. Especially behavioral characterization of animal models for relatively slow developing neurodegenerative diseases may benefit from automation of otherwise laborious longitudinal studies. The R6/2 mouse strain is a frequently used model for Huntington's Disease and we observed 28 R6/2 females (15 transgenic and 13 wildtype) for several weeks in a row in their home cage (PhenoTyper®, Noldus Information Technology, Wageningen, the Netherlands) and many aspects of their locomotion, circadian rhythm and feeding/drinking behavior were measured using EthoVision XT® software (Noldus Information Technology, Wageningen, the Netherlands). Mice had ad libitum access to food and water and a shelter was provided as a place to sleep and/or hide. Home cage monitoring was interrupted in order to weigh the mice, to measure grip strength and quantitatively assess footfalls and gait. Differences in grip strength and gait became apparent when animals were 8 weeks old. With home cage monitoring behavioral symptoms were already evident at the age of 4 weeks: transgenic animals showed an increase in the time spent inside the shelter, but a significant decrease in the time they spent on top of the shelter. Differences in locomotion were found as well, but only after dividing locomotion bouts into slow movements (lingering) and fast movements (progressing): compared to the wildtype, transgenic mice showed less fast movements. With aging the differences between transgenic and wild type mice became more and more pronounced. Finding differences already at the age of 4 weeks indicates that automatic home cage monitoring could be used as an early diagnostic tool and may be the answer to the pharmaceutical companies' need of better and faster animal models.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

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Program#/Poster#: 415.22/U15

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS043466

NIH F31 NS083289-01A1

Title: Structure/function analysis of the murine huntingtin N-terminus encoded by Htt exon 1 using knock-in mouse mutants

Authors: *E. ANDRE, J.-P. LIU, S. ZEITLIN
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Abstract: The polyglutamine (polyQ) stretch of mammalian huntingtin (htt) is flanked by a highly conserved 17 amino acid N-terminal domain (N17), and a proline-rich region (PRR). The PRR can modulate the aggregation properties of mutant htt N-terminal fragments, and is a binding site for many proteins that interact with htt. The N17 domain regulates several normal htt functions, including htt relocation into the nucleus in response to ER stress, and htt's association with organelles such as ER, endosomes, and autophagosomes. In addition, post-translational modification of amino acids within the htt N17 domain can affect normal htt subcellular localization, and can also regulate the toxicity and degradation of mutant htt. To explore further the contribution of the protein domains encoded by the *Htt* exon 1 to normal htt function, we have generated knockin mice expressing versions of htt lacking both the polyQ and PRR regions ($Htt^{\Delta QP}$), and mice expressing a version of htt lacking the N17 domain ($Htt^{\Delta N17}$). We have obtained $Htt^{\Delta QP/\Delta QP}$, and $Htt^{\Delta N17/\Delta N17}$ mice from heterozygous intercrosses at the expected Mendelian frequency, suggesting that deletion of the domains encoded by the *Htt* exon 1 do not affect htt's essential embryonic functions. We have completed characterizing these new models with respect to their motor function and performance in a spatial learning and memory test (Morris water maze, MWM). $Htt^{\Delta QP/\Delta QP}$ mutants exhibit improvements in motor coordination tasks but alterations in the MWM task that are dependent on the age of testing when compared to controls. $Htt^{\Delta N17/\Delta N17}$ mutants do not exhibit any differences in motor coordination in comparison to controls; however, these mutants do exhibit a phenotype in the MWM that is similar to that observed in $Htt^{\Delta QP/\Delta QP}$ mice. To determine if these deletions can affect HD pathogenesis *in trans*, we have also generated mice that express both a mutant version of htt with an expanded polyQ stretch, and either the N17 or QP domain deletion mutant versions of normal htt. At multiple ages, we have observed a rescue in MWM deficits in both the $Htt^{140Q/\Delta QP}$ and $Htt^{140Q/\Delta N17}$ mice in comparison to $Hdh^{140Q/+}$ mice, but deficits are observed in their motor coordination and activity levels in comparison to controls. We are currently characterizing these mice to determine if alterations in neuronal autophagy or in neuronal stress response occur.

Disclosures: E. Andre: None. **J. Liu:** None. **S. Zeitlin:** None.

Poster

415. Huntington's Disease Animal Models and Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Principal component analysis of fine motor skill impairment of R6/2 and zQ175 knock in mice of huntington's disease

Authors: *T. HEIKKINEN[1], T. BRAGGE[1], J. PUOLIVÄLI[1], A. NURMI[1], O. KONTKANEN[1], I. MUNOZ-SANJUAN[2], L. C. PARK[2]
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Abstract: In this study we implemented a principal component analysis (PCA) based method to create algorithms that would allow for data obtained from a high speed kinematic imaging analysis to show linear combinations of different pathological features that provides greater insight and sensitivity in the detection of fine motor skills in mice. Through implementation of these methods we have been able to establish the presence of progressive, earlier deficits in mouse models of Huntington's disease (HD). These deficits are observed in multiple combinations of different parameters describing gait properties and fine motor capabilities, at various ages in the mice and reveal linear combinations of different pathological features correlated to the disease progression with increased sensitivity in kinematic analysis. HD and wild-type mice, of both genders and at various ages were tested at various ages: walking, ladder walking and wading. The gait was recorded with a high speed camera, imaged and analyzed simultaneously from multiple viewing angles and utilizing comprehensive kinematic algorithms. Anatomical landmarks were marked with a contrast agent in order to enhance the detection of trajectories between particular landmarks in the images. A large number of parameters describing gait characteristics and fine motor movements were determined from the recognition of marker trajectories, and were compared between the genotypes to reveal disease specific defects. PCA was used to transform the parameter set into a new, smaller set of mutually uncorrelated parameters. In addition, PCA was applied directly to the marker trajectory data vectors, of which each were composed of referenced and interpolated marker data of individual gait cycles (, i.e., kinematic information of whole gait cycle). These results indicate that by employing PCA previously observed progressive HD-like fine motor deficits of R6/2 and zQ175KI mice can be further expressed as linear combinations of different parameters of fine motor movements. Moreover, the PCA shows stronger HD-phenotype and genotype difference than conventional parameter analysis, offering better power and a wider therapeutic window for possible treatment intervention in HD. Thus, the fine motor skill analysis using PCA offers a sensitive and perhaps more translatable tool in order to investigate the efficacy of therapeutic drugs that improve early and subtle motor functions in HD.

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Poster

415. Huntington's Disease Animal Models and Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Pfizer Inc., provided compounds and dose formulation information for this study

Title: Impact of phosphodiesterase 10A inhibition on spontaneous and cortically-evoked spike activity in the striatum of Q175 mice that model Huntington's disease

Authors: S. CHAKROBORTY[1], A. M. DEC[1], C. J. SCHMIDT[2], *A. R. WEST[1]
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Abstract: Huntington's disease (HD) is a genetic neurodegenerative disorder associated with abnormal expansion in CAG trinucleotide repeats within the Huntingtin gene. This genetic mutation induces the degeneration of striatal medium-sized spiny projection neurons (MSNs) via the synthesis of mutant huntingtin protein. It is now clear that one consequence of this mutation may be abnormalities in the metabolism of cyclic nucleotides by phosphodiesterases (PDEs). Specific findings point to decreased striatal cAMP/cGMP production, which may be linked to aberrant spontaneous discharge of MSNs and deficits in corticostriatal transmission. Thus, drugs designed to inhibit striatal PDE activity may be useful therapeutic agents for slowing disease progression and alleviating motor and cognitive symptoms of HD. The current study monitored spontaneous and cortically-evoked firing in aged (5-7 months old) wild-type (WT) and Q175 heterozygous knock-in mice treated with vehicle or the potent and selective PDE10A inhibitor PF-2545920. WT and Q175 mice were anesthetized with urethane and single-unit spike activity was isolated during low frequency electrical stimulation of the ipsilateral motor cortex. MSNs recorded in Q175 mice exhibited higher spontaneous firing rates than MSNs recorded in WT controls. The incidence of spontaneously firing MSNs was also significantly increased in Q175 mice, indicating that overall population activity is elevated in the HD striatum. Furthermore, MSNs recorded in Q175 mice exhibited significant decreases in cortically-evoked firing. Given this, it is likely that corticostriatal transmission is compromised in the HD striatum in a manner

that leads to increased background noise (spontaneous firing) and a decrease in the cortically-driven signal (i.e., decrease in the signal to noise ratio). Interestingly, PF-2545920 (1 mg/kg, s.c.) administration potently suppressed the spontaneous firing of MSNs recorded in Q175 mice to levels similar to that observed in WT controls. Less robust suppressive effects of PF-2545920 administration were also observed in WT controls. Preliminary observations from within-subjects recordings performed in Q175 mice revealed no significant differences in the probability of cortically-evoked spikes ~30 minutes after PF-2545920 administration. While more studies are needed to determine the impact of PF-2545920 administration on cortically-evoked activity, these observations indicate that PDE10A inhibition may partially normalize aberrant firing and population activity observed in the HD striatum and improve pathological disturbances in the above mentioned signal to noise ratio.

Disclosures: S. Chakroborty: None. **A.M. Dec:** None. **A.R. West:** None. **C.J. Schmidt:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 416.01/U18

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Kennedy's Disease Association

Title: Determining the role of ARD1 in SBMA

Authors: *H. L. MONTIE[1], S. HOSEIN[1], D. SIMPSON[1], W. LIU[2], D. E. MERRY[3], E. M. HEINE[1]
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Abstract: The goal of these studies is to determine the role of N-acetyltransferase arrest-defect 1 protein (ARD1, Naa10) in the aberrant acetylation, aggregation, and toxicity of polyglutamine (polyQ)-expanded androgen receptor (AR) in Spinal and Bulbar Muscular Atrophy (SBMA, Kennedy's disease). SBMA is an X-linked neuromuscular disorder, caused by the expansion of a polymorphic CAG tract ($n \geq 40$) in the coding region of the AR gene, which encodes a glutamine tract in the protein. SBMA is similar to other polyQ expansion diseases in that it presents in the third to sixth decade of life, the length of the polyQ tract is predictive of disease severity and

onset, and the disease associated protein aberrantly aggregates. Unlike the other polyQ expansion diseases, which are autosomal dominant, SBMA affects only men and is predominantly a lower motor neuron disease. There are currently no curative treatments for SBMA and this presents a critical challenge to define specific disease targets for therapeutic intervention. In previous studies, we have shown that polyQ-expanded AR is hyperacetylated and that AR acetylation modulates its nuclear aggregation and toxicity in cell models of SBMA. It has been recently reported that ARD1 enhances normal AR acetylation. Thus, we hypothesize that decreasing ARD1 protein levels or activity will reduce polyQ-expanded AR acetylation, aggregation, and toxicity in cell models of SBMA. To determine the effects that ARD1 has on these disease features, we are initially utilizing an inducible PC12 cell model of SBMA (AR112Q). Preliminary studies indicate that knockdown of ARD1 in these cells decreases AR112Q aggregation. We are currently evaluating whether decreased ARD1 also reduces polyQ-expanded AR acetylation and toxicity. We have also stably transfected AR112Q-expressing cells with exogenous ARD1 to determine the effect of overexpressed ARD1 on polyQ-expanded AR acetylation, aggregation and toxicity We propose that improved understanding of how polyQ-expanded AR acetylation may be regulated by ARD1 will lead to new therapeutic options for SBMA patients.

Disclosures: H.L. Montie: None. **W. Liu:** None. **D.E. Merry:** None. **E.M. Heine:** None. **D. Simpson:** None. **S. Hosein:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS03221

NIH Grant NS076919

Title: Identification of novel aggregation species in the polyglutamine disease spinal and bulbar muscular atrophy

Authors: *T. R. BERGER[1], P. JAIN[2], J. LEGLEITER[2], A. PLUCIENNIK[1], L. ZBORAY[1], E. HEINE[3], H. MONTIE[3], D. MERRY[1]

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Abstract: Polyglutamine-repeat disorders are part of a larger family of neurodegenerative diseases characterized by protein misfolding and aggregation. In spinal and bulbar muscular atrophy (SBMA), polyglutamine expansion within the androgen receptor (AR) causes progressive debilitating muscular atrophy and lower motor neuron loss in males. Soluble aggregates of the AR are considered a toxic intermediate in the aggregation process. Here, we utilized SDS-agarose gel electrophoresis, sedimentation analysis, and atomic force microscopy to identify a novel aggregation species of the polyglutamine-expanded AR - one that is soluble, consists of full-length AR, and binds the toxicity-predicting antibody 3B5H10. Disease relevance was confirmed *in vivo* and correlations were made to toxicity based on a library of well-characterized structural and functional mutations of the polyglutamine-expanded AR. Identifying a common signature of soluble AR species will advance the development of targeted therapies applicable to a wide range of neurodegenerative diseases caused by protein misfolding.

Disclosures: T.R. Berger: None. **P. Jain:** None. **J. Legleiter:** None. **A. Pluciennik:** None. **L. Zboray:** None. **E. Heine:** None. **H. Montie:** None. **D. Merry:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

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Program#/Poster#: 416.03/U20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS045195

Title: SBMA motor dysfunction may be due to failed neuromuscular transmission

Authors: Y. XU[1], W. ATCHISON[1], H. ADACHI[4], M. KATSUNO[5], G. SOBUE[6], S. BREEDLOVE[2], *C. L. JORDAN[3]
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Abstract: Spinal and bulbar muscular atrophy (SBMA) affects only men, is androgen-dependent, and linked to an expanded polyglutamine (polyQ) tract in the androgen receptor (AR). While traditionally viewed as neurogenic, data from a novel "myogenic" mouse model suggest that SBMA is driven by AR toxicity originating in skeletal muscle (Monks et al., *PNAS* (2007) 104:18259), a notion that has recently been confirmed in other models (*Neuron* (2014) 82:295; *Cell Report* (2014) 7:1). However, whether muscle or motoneuron dysfunction underlies motor dysfunction remains unknown. While muscle strength is impaired in SBMA mice, no data exist about whether disease also affects neuromuscular synaptic strength. Thus, we recorded spontaneous (miniature) and evoked endplate potentials (mEPPs and EPPs, respectively) intracellularly from healthy and diseased muscle fibers of three different mouse models of SBMA: the "myogenic" model which overexpresses a wild-type (wt) AR exclusively in skeletal muscles, the "AR97Q" model that globally expresses a full length human AR with 97 polyQs, and a knockin (KI) model where the first exon of the endogenous *AR* is replaced by the human exon with 113 CAGs. We find diseased synapses are appreciably weaker, likely due to a lower release probability of synaptic vesicles. For example, quantal content (QC), an indicator of the amount of acetylcholine (ACh) released in response to action potentials, was significantly reduced (30-40%) in all three SBMA models. We also find decreases in both size of the readily releasable synaptic vesicle pool (RRP) and in short term synaptic facilitation. Interestingly, in early symptomatic KI mice, the RRP size was unaffected but short term synaptic facilitation was increased, suggesting reduced release probability might be an early cause of reduced ACh release. Disease also affected postsynaptic mechanisms: resting membrane potential of diseased fibers was depolarized by ~10-15 mV (relative to wt controls) and associated with a resistance to μ-conotoxin, a selective blocker for skeletal muscle sodium channels. Increased amplitude of mEPPs, and prolonged decay times for both mEPPs and EPPs, suggest SBMA also impairs expression of several critical ion channels in muscle. Together, these data show comparable defects in neuromuscular transmission across three *different* SBMA mouse models, involving both pre- and post-synaptic mechanisms. That the *same defects* are seen in the muscle-specific model as in the other two models argues that AR toxicity emanating from muscle imparts dysfunction to *both* muscles and motoneurons and that wt AR is capable of engaging the same toxic mechanisms as polyQ-expanded AR.

Disclosures: **Y. Xu:** None. **C.L. Jordan:** None. **W. Atchison:** None. **M. Katsuno:** None. **H. Adachi:** None. **G. Sobue:** None. **S. Breedlove:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

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Program#/Poster#: 416.04/U21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS04519

Title: Androgen-dependent deficits in muscle-derived BDNF correlate with motor dysfunction in two mouse models of spinal bulbar muscular atrophy

Authors: *K. HALIEVSKI[1], Y. XU[1], C. L. HENLEY[1], M. KATSUNO[2], H. ADACHI[3], G. SOBUE[2], S. M. BREEDLOVE[1], C. L. JORDAN[1]
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Abstract: Spinal bulbar muscular atrophy (SBMA) is a late-onset, progressive neuromuscular disease linked to a polyglutamine expansion mutation in the androgen receptor (AR) gene. SBMA is also androgen-dependent, requiring male levels of testosterone for expression of the disease. Target derived neurotrophic factors, including brain-derived neurotrophic factor (BDNF), are critical for the survival and function of neurons. We previously reported a reduction of BDNF mRNA in skeletal muscles of symptomatic mice in two different transgenic SBMA models: 1) the myogenic model that overexpresses wild-type rat AR in skeletal muscle fibers, and 2) the AR97Q model that globally overexpresses a full length human AR with 97 glutamine repeats. We now show that the deficit in BDNF mRNA expression in diseased muscle is androgen-dependent, and thus, tightly correlates with motor dysfunction, and is independent of transgene expression per se. In the myogenic model, castration of chronically diseased males greatly improves motor performance, which is paralleled by a significant increase in BDNF mRNA expression in muscle. Furthermore, when asymptomatic myogenic females are treated with testosterone, their motor function quickly plummets, reaching male levels of motor dysfunction in only five days of treatment. Likewise, the level of BDNF mRNA in skeletal muscle of acutely diseased myogenic females drops to that of chronically diseased males within that time. Ongoing experiments in our laboratory are characterizing the time-course of this BDNF reduction in acutely diseased myogenic females to determine whether the deficit in BDNF mRNA expression precedes the onset of motor dysfunction, supporting the idea that BDNF is an important regulator of motor function. In the second, AR97Q model, we find comparable androgen-dependent deficits in BDNF expression in diseased muscle. Castrating AR97Q males at P28-32 prevents the decline in both motor function and BDNF expression in muscle that occurs when AR97Q males remain gonadally intact. In sum, we find BDNF mRNA deficits in SBMA muscle are dynamically regulated by androgen levels and change in step with the quality of motor function. Moreover, these data suggest that reduced BDNF levels may underlie motor dysfunction in SBMA and raise the question of whether replenishing the system with BDNF could rescue function in mouse models of SBMA. This work was funded by NIH NS04519 (CLJ).

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Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 416.05/U22

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Kennedy's Disease Association

Title: The role of Tip60, an androgen receptor acetyltransferase, in SBMA

Authors: ***E. HEINE**[1], C. N. ROBSON[2], D. E. MERRY[3], H. L. MONTIE[1]
[1]Philadelphia Col. of Osteo. Med., PHILADELPHIA, PA; [2]Newcastle Univ., Newcastle upon Tyne, Tyne and Wear, United Kingdom; [3]Thomas Jefferson Univ., Philadelphia, PA

Abstract: Spinal and Bulbar Muscular Atrophy (SBMA; Kennedy's Disease) is an adult-onset, slowly-progressive, X-linked neuromuscular disease. SBMA results from a CAG repeat expansion in the coding region of the androgen receptor (AR) gene, leading to a polyglutamine (polyQ) repeat expansion in the amino-terminal domain of the AR protein. Patients are primarily male, both due to random X-inactivation as well as the disease requirement of high circulating levels of androgens, and present with weakness and atrophy of muscles of the limbs, mouth, and throat. These symptoms result from the loss of lower motor neurons in the spinal cord and brainstem and due to muscle atrophy. A histopathological hallmark of SBMA, as well as other polyQ-expansion diseases, is the presence of intranuclear inclusions composed mainly of amino-terminal portions of the polyQ-expanded AR protein. To date, there is no curative treatment or effective therapy for SBMA. Recently, we have shown that hormone-dependent AR acetylation at K630/632/633 is a major modifier of polyQ-expanded AR toxicity and inclusion formation. Tip60 (Tat-interactive protein, 60kDa) functions as a histone acetyltransferase (HAT) and has been shown to enhance AR transactivation by directly interacting with and acetylating K630/632/633. Here, we are studying the role of Tip60 in SBMA by investigating the loss of Tip60 protein or pharmacological inhibition of its acetyltransferase activity. Transient knock-down of Tip60 in a PC12 cell model of SBMA reduced the formation of nuclear inclusions of polyQ-expanded AR. Clonal cell lines are currently being screened for Tip60 knock-down and will be used to further study the affect of Tip60 on AR acetylation, aggregation and toxicity. In addition to genetic knock-down of Tip60, we have found that pharmacological inhibition of this

enzyme abrogates polyQ-expanded AR aggregation in PC12 cells. Future studies will assess the mechanism of Tip60's role in the pathogenic mechanisms of SBMA as well as to determine the therapeutic potential of inhibiting Tip60 *in vivo*. The ultimate goal of these studies is to identify whether Tip60 is a valid therapeutic target for the treatment of SBMA.

Disclosures: E. Heine: None. **H.L. Montie:** None. **D.E. Merry:** None. **C.N. Robson:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 416.06/U23

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R56 NS032214

Title: Analysis of nuclear export of polyglutamine-expanded androgen receptor in a cell model of spinal and bulbar muscular atrophy

Authors: F. ARNOLD[1], H. MONTIE[2], *D. E. MERRY[1]
[1]Dept Biochem & Molec Biol, Thomas Jefferson Univ., PHILADELPHIA, PA; [2]Philadelphia Col. of Osteo. Med., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA) is an X-linked neurodegenerative disease caused by a polyglutamine (polyQ) expansion in the androgen receptor (AR) protein. The AR is a steroid hormone receptor that, upon ligand binding of testosterone or dihydrotestosterone (DHT), undergoes a conformational change that induces nuclear localization of the AR and subsequently, the transcriptional regulation of AR-target genes. In SBMA, both the presence of hormone and the nuclear localization of the AR are necessary for toxicity, with the formation of intranuclear inclusions of aggregated AR a hallmark of the disease state. Given the importance of nuclear localization in disease-mediated toxicity, we sought to examine whether there are alterations in the nuclear export of polyQ-expanded AR, compared with wild-type AR and known AR export-deficient mutants. To test the hypothesis that polyQ-expansion impairs nuclear export of the AR, we developed a heterokaryon assay using a well-characterized PC12 SBMA cell model and mouse fibroblast (NIH/3T3) cells. In these experiments, nuclear localization of the AR is induced in PC12 cells by a short hormone treatment to avoid nuclear inclusion formation in cells expressing polyQ-expanded AR. Concurrently, protein synthesis is inhibited with cyclohexamide. Heterokaryons are then formed by fusing PC12 and NIH/3T3 cells; after

cell fusion, any AR detected in an NIH/3T3 cell nucleus must first have been exported from a PC12 cell nucleus. Results of these studies suggest that polyQ-expansion in the AR protein impairs nuclear export, as 55% of heterokaryons formed with PC12 cells expressing polyQ-expanded AR had either no detectable AR in the NIH/3T3 cell nucleus, or significantly more AR in the PC12 cell nucleus than in the NIH/3T3 cell nucleus. On the other hand, 100% of heterokaryons formed with PC12 cells expressing wild-type AR had detectable AR in the NIH/3T3 cell nucleus and only 11% of these heterokaryons had significantly more AR in the PC12 cell nucleus than the NIH/3T3 cell nucleus. These data highlight a novel metabolic deficit inherent in polyQ-expanded AR and the importance of further studying the role of nuclear export in SBMA disease pathogenesis.

Disclosures: F. Arnold: None. **D.E. Merry:** None. **H. Montie:** None.

Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 416.07/U24

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: A pharmacological approach to induce the clearance of mutant androgen receptor in spinal and bulbar muscular atrophy

Authors: *P. RUSMINI, E. GIORGETTI, V. CRIPPA, R. CRISTOFANI, M. CICARDI, A. POLETTI
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Abstract: Spinal and bulbar muscular atrophy (SBMA) is a motoneuronal disease caused by the expansion of a CAG repeat in the coding region of the Androgen Receptor (AR) gene, resulting in a protein with an abnormally elongated polyglutamine (polyQ) tract. The binding of the physiological ligand testosterone to the mutant protein induces ARpolyQ misfolding and aggregation in lower motoneurons. These aggregates colocalized with several molecular chaperones, and components of the ubiquitin-proteasome system (UPS) and the autophagic pathways, involved in protein quality control system. This suggests the possibility that the degradative pathways may be actively involved in the clearance components of these inclusions but they fail to completely degrade the ARpolyQ protein. In particular, cytoplasmic ARpolyQ aggregation seems to depend on autophagic flux blockage. We have already demonstrated that the anti-androgen, Bicalutamide, is able to retain the activated ARpolyQ for a long time period

into the cytoplasm without triggering aggregate formation. Moreover, trehalose, an autophagic inducer, is able to induce the ARpolyQ clearance through this cytoplasmic degradative pathway. We have tested the combined effects of these two compounds. Bicalutamide and trehalose co-treatment decreased the ARpolyQ accumulation and had a more potent effect in reducing the soluble and insoluble forms of ARpolyQ than the single treatments. Analysing the autophagic markers LC3 and p62, we observed that trehalose strongly actived the autophagic pathway while casodex had no effects. Interestingly, Bicalutamide and trehalose co-treatment, is also able to induce the clearance of soluble and insoluble forms of a long expanded polyQ tract (ARQ112), which led to the formation of nuclear aggregates. These data suggest that Bicalutamide and trehalose co-treatment offers the opportunity to retain into cytoplasm the activated ARpolyQ for a long period improving the effects of trehalose-induced autophagic clearance, leading to a more efficient removal of ARpolyQ. These results might represent a possible therapeutic approach for SBMA. GRANTS: Fondazione AriSLA; AFM Telethon France; Regione Lombardia multicentric project; UNIMI

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Poster

416. SBMA and Other Non-Huntington's Disease Repeat Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Les Turner ALS Foundation

Les Turner ALS Foundation/Herbert C. Wenske Foundation Professorship

Foglia Family Foundation

Ride for Life

Title: Study of intermediate-length polyQ repeats in sporadic amyotrophic lateral sclerosis

Authors: *F. L. NUNEZ SANTANA, N. A. SIDDIQUE, T. SIDDIQUE
The Ken & Ruth Davee Dept. of Neurol., Northwestern University, Feinberg Sch. Med., Chicago, IL

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive, paralytic, and ultimately fatal neurodegenerative disease characterized by motor neuron degeneration. Approximately 10% of ALS cases have a familiar origin (FALS) with the remainder of cases being sporadic (SALS). To date, disease mechanisms for both FALS and SALS have yet to be elucidated; however, about 20% of FALS cases are known to occur from mutations in the Cu, Zn Superoxide dismutase 1 (SOD1) gene (Rosen et al. 1993). An ongoing challenge in the study of ALS is that its pathogenesis is largely unknown. Nonetheless, recent studies have shown a significant association between intermediate-length polyQ repeats (CAG) in the ataxin-2 gene (ATXN2) and genetic risk factor for ALS (Elden et al. 2010). In other words, it appears that depending upon the length of the repeat expansions in ATXN2, individuals can present a higher risk for development of the disease. However, the overall number/range of intermediate or fully pathological repeats associated with ALS have been found to vary among studies. We have analyzed the size of ATXN2 CAG repeats from SALS and healthy age/race matched control patients, with aims of refining the range of intermediate and/or pathological repeat number significantly associated with SALS. The ATXN2 repeat regions from genomic DNA samples were amplified by polymerase chain reaction using Taq Gold Polymerase in the presence of fluorescent forward and reverse primers. PCR fragment analysis and number of CAG repeats were determined after capillary electrophoresis using a Beckman Coulter CEQ 8000 Genetic DNA Analysis Sequencer System. As previously reported, the length of the normal allele for SALS patients was found to be between 20-22 CAG repeats, whereas, the longer alleles in SALS patients ranged from 27 to 31 repeats (2.41%). Controls patients ranged from 27 to 28 repeats (0.48%). Our preliminary findings further confirm an association between ATXN2 intermediate CAG expansions and increased risk for ALS. However, given the overlap between SALS and control patient repeat expansions, our preliminary data indicate that individuals with repeat expansion of 29-33 may carry a bigger risk for development of ALS.

Disclosures: F.L. Nunez Santana: None. **N.A. Siddique:** None. **T. Siddique:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 417.01/U26

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MURST (PRIN project 2006058401)

Title: The modulation of glutamate release by pre-synaptic group 1 metabotropic glutamate receptors in als

Authors: *C. USAI[1], M. MILANESE[2,3], T. BONIFACINO[2,3], P. I. A. ROSSI[4,5], A. PULITI[4,5], A. PITTALUGA[2,3], G. BONANNO[2,3]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease characterized by muscle wasting, weakness and spasticity, reflecting a degeneration of upper and lower motor-neurons (MNs). The mechanisms of neuronal death in ALS are still largely obscure. It is well known that Glutamate(Glu)-mediated excitotoxicity plays a major role in the degeneration of motor neurons. According to our studies, we suggested that the high levels of synaptic Glu are due, not only to a reduced astrocytary transport (1), but also to an abnormal release (2). Recently, pre-synaptic mGlu1 and mGlu5 receptors were described in rat cerebral cortex nerve terminals, whose activation produced potentiation of Glu release (3). In the present work we investigated on the presence of similar autoreceptors in the spinal cord of SOD1/G93A mice, a widely used animal model of human ALS. Exposure of spinal cord synaptosomes to increasing concentrations of the mGluR1/5 agonist 3,5-DHPG produced distinct effects in SOD1/G93A mice and controls: concentration above 0.3 μM stimulated the basal release of [3H]D-Aspartate, used to label the endogenous pools of Glu, both in control and SOD1/G93A mice. At variance, concentrations of 3,5-DHPG equal to or lower than 0.31 μM increased [3H]D-Asp release in SOD1/G93A mice, only. Experiments with selective mGluR1 or mGluR5 antagonists indicated that the both high and low concentration effects of 3,5-DHPG involved mGluR1 and mGluR5 activation. Low 3,5-DHPG concentrations induced increase of IP3 in SOD1/G93A but not in control mice; whereas, high 3,5-DHPG induced IP3 formation in both mouse strains. Release experiments confirmed that 3,5-DHPG produced exocytotic release of [3H]D-Aspartate, involving intra-terminal Ca2+ release through IP3-sensitive channels. Protein and mRNA determination pointed towards a more elevated expression of mGluR5 in SOD1/G93A mice. Overall our results demonstrate the existence of an abnormal mGluR1/5-mediated release of Glu in SOD1/G93A mice, which may represent a cause of the excessive Glu levels. This phenomenon can be modulated by selective antagonists, confirming an interplay between the two receptors and an involvement in ALS. These data provide a rationale for new pharmacological approaches based on the selective block of Group I mGluRs. (1) Rothstein JD, Van Kammen M, Levey AI et al Ann. Neurol. 1995; 38(1):73-84 (2) Milanese M, Zappettini S, Onofri F et al J. Neurochem. 2011; 116(6):1028-1042 (3) Musante V, Neri E, Feligioni M et al., Neuropharmacology. 2008 Sep;55(4):474-82

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 417.02/U27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Induction of NF-KB activation by ALS-linked Ubiquilin-2 mutant

Authors: *V. PICHER-MARTEL, A. AYOUAZ, D. PHANEUF, J.-P. JULIEN
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Abstract: Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor disease and is characterized by progressive death of upper and lower motor neurons. This degeneration leads to progressive paralysis of skeletal muscle and, unfortunately, to patient's death within 2-5 years of symptoms. Most of ALS cases are sporadic (90%) and only 5-10% are familial. In familial cases, some gene has been linked to the pathology like superoxide dismutase 1 (SOD1) (20%), TAR DNA-binding protein (TDP-43), FUS, P62/SQSTM1 or C9ORF72. Ubiquilin-2 (UBQLN2) plays an important role in ubiquitin proteasome system (UPS) and autophagy by connecting the UPS and ubiquinated protein. Recently, an X-linked mutation in UBQLN2 gene has been discovered in (ALS) familial cases. Approximately ten mutations have been identified and the main one is P497H. These patients developed cytoplasmic inclusions positive for major proteins implicated in this neurodegenerative disorder and also show UPS impairment. Furthermore, ALS patients without UBQLN2 mutation also express UBQLN2 positive inclusions, supporting an important role of this protein in ALS physiopathology. There is an emerging role of nuclear factor kappaB (NF-κB) in ALS and other neurologic diseases. For example, it has been shown in our lab that TDP-43 upregulation can enhance activation of NF-κB. We used cell cultures to determine if UBQLN2 mutation and accumulation induce NF-κB activation in ALS pathology. Neuro2A cells, neurons derived from mouse, were stably transfected with NF-κB activation luciferase reporter and then with UBQLN2 WT or P497H plasmids for 48 hours. Luciferase activity and western analysis show an increase in NF-κB activation in cells overexpressing UBQLN2 P497H compare to non-transfected and UBQLN2 overexpressing cells. These inclusions also seem to be related to NF-κB pathway proteins, which can explain this activation.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: Dysregulated gene expression in the axotomized facial motor nucleus of RAG-2 KO mice: Relevance to ALS

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Abstract: In the mouse, the immune system plays an important role in promoting facial motoneuron (FMN) survival after facial nerve transection at the stylomastoid foramen. In wild type (WT) mice, 87% of FMNs survive axotomy, whereas in recombination-activating gene-2 knockout (RAG-2 KO) mice, only 66% of FMNs survive. RAG-2 KO mice lack the adaptive arm of the immune system, causing them to be immunodeficient. In the present study, we examined how the immune system promotes motoneuron survival by analyzing gene expression changes in axotomized WT and RAG-2 KO mice using laser capture microdissection and qPCR. First, we studied the ability of FMNs to regenerate by studying expression of growth-associated protein 43 (GAP-43), which is a key molecule expressed by FMNs and is involved in axonal regeneration and target reconnection. Interestingly, both RAG-2 KO and WT FMNs significantly upregulate GAP-43 in response to injury. Next, we studied the surrounding microenvironment by assessing astrocyte and microglia response to axotomy. When glial fibrillary acidic protein (GFAP) and CD68 expression levels were measured, the RAG-2 KO facial motor nucleus had a significantly reduced activation response relative to WT. This result suggests an impairment in the astrocytic/microglial response to peripheral axotomy in immunodeficient mice. Another molecule expressed in the microenvironment surrounding the FMN after axotomy is tumor necrosis factor-α (TNFα), a key signaling molecule of the immune system. In WT mice,

expression of TNFα increased 10-fold in the axotomized facial motor nucleus for the first two weeks after injury, and then subsequently decreased to below detectable levels. In RAG-2 KO mice, the increased TNFα levels observed within 2 weeks post-axotomy were approximately half of WT levels. These data support a role for TNFα in neuroprotection. Expression of beta-2 microglobulin (B2M), a component of major histocompatibility complex I (MHC I), was also significantly decreased in the axotomized facial motor nucleus of RAG-2 KO mice relative to WT. Collectively, these data suggest that in the RAG-2 KO mouse, a dysregulated microenvironment involving significant suppression of both astrocytic and microglial responses to peripheral axotomy plays a role in increased FMN death with injury. In contrast, the intrinsic neuronal repair process appears unaffected. Comparing these gene expression results to those of SOD1-G93A mice reveals striking similarities, as this mouse model of ALS also demonstrates a strong neuronal regenerative response but deficient astrocytic and microglial response to facial nerve axotomy.

Disclosures: D.N. Olmstead: None. **N.A. Mesnard-Hoaglin:** None. **M.M. Haulcomb:** None. **R.J. Batka:** None. **N.D. Schartz:** None. **V.M. Sanders:** None. **K.J. Jones:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Title: CIIA prevents SOD1(G93A)-induced cytotoxicity by blocking ASK1-mediated signaling

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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease with higher selectivity in degeneration of motor neurons. However, the molecular mechanism by

which the ALS-linked mutants of human superoxide dismutase 1 (SOD1) gene induce neurotoxicity remains obscure yet. Here, we show that depletion of CIIA expression by RNA interference (RNAi) promoted cytotoxicity caused by ALS-linked G93A mutant of the SOD1 gene. The RNAi-mediated knockdown of CIIA also enhanced the SOD1(G93A)-induced interaction between ASK1 and TRAF2 as well as ASK1 activity. Furthermore, endogenous silencing of CIIA by RNAi augmented the effects of SOD1(G93A) on reduction of mitochondria membrane potential ($\Delta\psi m$), release of cytochrome c into the cytoplasm, and caspase activation. Together, our results suggest that CIIA negatively modulates ASK1-mediated cytotoxic signaling processes in a SOD1(G93A)-expressing cellular model of ALS.

Disclosures: **J. Lee:** None. **S. Hwang:** None. **J. Shin:** None. **J. Shim:** None. **E. Choi:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: DFG BR3706/3-1

Title: Linking mitochondrial and autophagic activities in neurotoxic yeast cell death models

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Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with progressive dysfunction and death of motor neurons in brain and spinal cord. The loss of neurons results in muscular weakness and atrophy, speech deficits and death due to respiratory paralysis. Mutations in the genes C9orf72, SOD1, FUS/TLS, and TDP-43 have been associated with ALS. The RNA-binding protein TDP-43 (TAR DNA-binding protein) has been found to accumulate in ubiquitinated, phosphorylated and insoluble inclusions in the cytoplasm of neurons of ALS patients. Mutations of TDP-43 cause similar pathophysiological effects in different model systems, such as mammalian cell cultures, flies and yeast. Saccharomyces cerevisiae is an established model for TDP-43 proteinopathies and many other neurotoxic diseases. Previous works revealed a connection between mitochondrial activity and TDP-43 cytotoxicity. Here, we investigate the link between the cytotoxicity of TDP-43, mitochondrial and autophagic activities in yeast. Methods and Results: We used the drop dilution assay to determine the cytotoxicity of

TDP-43 by measuring growth deficiency. Therefore, yeast wild type (wt) and atg (autophagy related) knock-out strains were transformed with expression constructs encoding human TDP-43 or vector control. The growth media were supplemented with different carbon sources (e.g., Glc and Gal) to modulate mitochondrial respiratory activities. In order to increase autophagy, growth media contained rapamycin blocking the TORC1 (target of rapamycin complex 1) signalling pathway. Expression of human TDP-43 causes cytotoxicity in yeast. We observed enhanced TDP-43 cytotoxicity with increased respiratory capacity and with increased autophagy, respectively. TDP-43 cytotoxicity was relieved by deletion of ATG genes. atg1, atg11 and atg15 are examples. Thus, autophagy seems to be important for the cytotoxicity of TDP-43. We used the GFP-Atg8 processing assay to study autophagic turnover upon TDP-43 expression. By transforming wt and atg1 with TDP-43 and GFP-Atg8, we examined the level of autophagy, which is indicated by the degree of free GFP. We observed an increase of processed GFP by expression of TDP-43 during starvation and non-starvation conditions. Thus, TDP-43 increased the level of autophagy. Conclusions: Our preliminary results suggest that mitochondria and autophagy influence TDP-43-triggered cytotoxicity. We believe that insights gained by applying yeast as cell death model promote a better understanding of the relation between autophagy and mitochondrial activities in ALS.

Disclosures: C. Leibiger: None. **R. Braun:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Herbert C. Wenske Foundation

Title: Visualization of CSMN in the absence of Alsin function in AlsinKO-UeGFP mice reveals details of cellular vulnerability

Authors: *M. GAUTAM[1], G. SEKERKOVA´[2], M. V. YASVOINA[1], J. H. JARA[1], M. MARTINA[2], P. H. OZDINLER[1]
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Abstract: Corticospinal motor neurons (CSMN) are unique in their ability to collect, integrate, translate and transmit cerebral cortex's input towards spinal cord targets. However, they are not easy to study due to the complexity and the heterogeneous nature of the cerebral cortex. Their degeneration is key in numerous neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Mutations in the Alsin 2 (ALS2) gene are responsible for juvenile primary lateral sclerosis, infantile onset ascending hereditary spastic paraplegia, and are the most common cause for autosomal recessive juvenile ALS. In addition, upper motor neuron signs and bulbar symptoms are often prevalent in patients with juvenile ALS. Here, we investigated the health, stability and cellular vulnerability of CSMN in the absence of Alsin protein using Alsin KO and the novel reporter line, the AlsinKO-UeGFP mice, which is generated by crossbreeding Alsin KO with the UCHL1-eGFP mice. In the WT-UeGFP and the AlsinKO-UeGFP mice, the CSMN are genetically labeled with eGFP that lasts through adulthood and late ages, allowing visualization and cellular analysis of CSMN at P300, P500 and beyond. Our ongoing studies suggest very subtle, yet important cellular changes that occur in CSMN. There is axonal degeneration of the subcerebral projection neurons, including CSMN, increased autophagy with age and changes in the localization of autophagic vesicles toward apical dendrites. Even though the neurons are not completely cleared from the motor cortex, detailed cellular visualization and analysis using immunocytochemistry coupled with electron microscopy (EM) reveal lack of health. Investigation of pure upper motor neuron defects in mouse is challenging, but here we demonstrate that using UCHL1-eGFP mice as a reporter for CSMN, their health and potential pathways that contribute to their vulnerability can be studied at a cellular level with high precision.

Disclosures: M. Gautam: None. **G. Sekerkova´:** None. **M.V. Yasvoina:** None. **J.H. Jara:** None. **M. Martina:** None. **P.H. Ozdinler:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Title: Mutant SOD1 misfolding and ER dysfunction in the pathogenesis of familial ALS

Authors: *H. KAWAMATA
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Abstract: Protein misfolding and the formation of insoluble aggregates containing multiple proteins are pathological disease hallmarks of many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Mutations in superoxide dismutase 1 (SOD1) are associated with autosomal dominant familial ALS. SOD1 is a soluble and ubiquitous antioxidant protein, which, when mutated, forms intracellular aggregates and cytoplasmic inclusions with toxic functions. Through cell fractionation experiments, SOD1 aggregates have been identified in various subcellular compartments of mutant cells, including the endoplasmic reticulum (ER)[1]. The ER is involved in a multitude of cell functions including calcium homeostasis, lipid biosynthesis, and protein folding and secretion. The ER protein folding machinery involves a series of coordinated redox relay systems. Misfolding of proteins in the ER causes a burden for the ER protein folding machinery, which cause activation of the unfolded protein response (UPR) and ER-associated degradation to regain cellular homeostasis. If the ER cannot cope with this stress, the UPR eventually activates apoptotic cell death mechanisms. UPR activation is a pathological hallmark in neurodegenerative conditions. However, the relationship among misfolded mutant SOD1, UPR and ER stress, and disease pathogenesis remains unknown. Recently, we discovered the role of mutant SOD1 in enhancing redox protein modification of the ER resident protein STIM1, and resulting alterations in store operated calcium entry and intracellular calcium dynamics. Importantly, abnormal STIM1 redox modifications and intracellular calcium abnormalities were ameliorated by supplementation of the redox agent glutathione[2]. Here, we show that mutant SOD1 expression in cells causes enhanced protein folding rate and that the ER environment is more oxidative as compared to wild type SOD1 expressing cells. The consequences of mutant SOD1 misfolding on ER function and the downstream pathogenic pathways are being investigated. [1] Urushitani M et al. FASEB J. 2008, 22(7): 2476-87 [2] Kawamata H et al. J Neurosci. 2014, 34(6): 2331-48

Disclosures: H. Kawamata: None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Title: Nuclear TDP-43 induces neuronal cell death by associating with heterogeneous nuclear ribonucleoprotein-U

Authors: *H. SUZUKI, M. MATSUOKA
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Abstract: Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease, characterized by a selective loss of upper and lower motor neurons. Frontotemporal lobar degeneration (FTLD) is characterized by the degeneration of the frontal and anterior temporal lobes. It is currently thought that the pathogenesis of these neurodegenerative diseases is at least partially linked to dysfunction of transactive response DNA-binding protein-43 (TDP-43) since TDP-43 has been identified as a major component of ubiquitinated inclusions in most cases of ALS and some cases of FTLD and a variety of mutations in TARDBP, the gene encoding TDP-43, have been identified in ALS and FTLD. TDP-43 predominantly localizes in nucleus (nuclear TDP-43) and a portion of TDP-43 forms aggregation in cytoplasm (cytoplasmic TDP-43) in the affected area of ALS and FTLD patients. It remains unknown which of nuclear and cytoplasmic TDP-43 exerts neurotoxicity. In this study, we first found that nuclear TDP-43 is more toxic to neurons than cytoplasmic TDP-43. To further investigate the molecular mechanism underlying TDP-43-induced neuronal cell death, we searched for TDP-43 interactors by GST pull down assay and mass-spectrometry analysis and identified heterogeneous nuclear ribonucleoprotein-U (hnRNP-U) as a TDP-43 interactor. The siRNA-mediated reduction of hnRNP-U expression induced cell death. Reciprocally, overexpression of hnRNP-U attenuated TDP-43-induced cell death by binding to TDP-43. These data together suggest that nuclear TDP-43 exerts neuronal toxicity and hnRNP-U negatively regulates TDP-43 neuronal toxicity.

Disclosures: H. Suzuki: None. **M. Matsuoka:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Title: The role of ephrin-b2 in amyotrophic lateral sclerosis

Authors: L. RUE[1], L. SCHOONAERT[1], L. POPPE[1], M. TIMMERS[1], A. VAN HOECKE[2], P. VAN DAMME[1,3], R. LEMMENS[1,3], *W. L. ROBBERECHT[3,1]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects lower motor neurons in brainstem and spinal cord, and the upper motor neurons in the motor cortex, and leads to a progressive muscle phenotype in patients. ALS is characterized by considerable genetic heterogeneity since mutations in more than 10 different genes (eg SOD1, FUS, TDP, C9ORF72) are known to cause the hereditary form of ALS. Similar heterogeneity is observed in the clinical presentation. This indicates that there are factors that modify the phenotypic expression of the disease. The tyrosine kinase receptor EphA4 was recently shown to be a modifier of ALS. Genetic and pharmacological inhibition of EphA4 rescued the phenotype in a zebrafish model of ALS and increased survival in ALS rodent models. In ALS patients an inverse correlation was found between EphA4 expression and disease onset. However, the mechanism of action has not yet been fully elucidated. EphA4 interacts with ephrin-a and ephrin-b ligands. Several of these EphA4 interaction partners have been shown to be not only expressed on motor neurons, but also on astrocytes, microglia and oligodendrocytes. These cells surrounding the motor neurons play an important role in the pathogenesis of ALS. Here, we aimed to determine the contribution of these various cell types and one specific EphA4 ligand, ephrin-b2, in ALS disease progression. First we performed immunofluorescence stainings of ephrin-b2 in the spinal cord of an ALS mouse model, overexpressing mutant SOD1 (SOD1G93A) and compared this pattern to mice overexpressing wild-type SOD1 (SOD1WT) at different stages of the disease. In SOD1WT spinal cord we observed ephrin-b2 to be highly expressed in motor neurons and oligodendrocytes, while only faint expression was detected in astrocytes. In symptomatic SOD1G93A spinal cord the expression pattern of ephrin-b2 in astrocytes and motor neurons changed. Immunoreactivity was markedly upregulated in astrocytes, but the presence of ephrin-b2 was clearly reduced in the neuronal population. As the expression pattern changed in the different cell types with disease progression, we next explored a possible modifying cell-specific role of ephrin-b2 in ALS, by generating a conditional ephrin-

b2 knockout mouse, in which ephrin-b2 is deleted upon GFAP expression. Deleting ephrin-b2 in reactive astrocytes of the SOD1G93A ALS mouse model resulted in a delay of disease onset and prolonged disease duration. These results suggest astrocytic ephrin-b2 to play a role in modifying ALS. In future experiments we intend to further explore the cellular mechanism of ephrin signalling in the pathophysiology of motor neurodegeneration.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Robert Packard Center for ALS Research

Title: Pathways of degradation of dysfunctional mitochondria in NSC34 motor neurons expressing mutant SOD1

Authors: G. M. PALOMO[1], J. MAGRANE[1], I. SHAHI[1], *G. MANFREDI[2]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal disease caused by the degeneration of upper and lower motor neurons. Familial forms of ALS represent a 10% of the all cases, and mutations in the gene Cu-Zn superoxide dismutase (SOD1) account for 20% of familial forms. Numerous molecular mechanisms have been proposed to explain the neuronal degeneration in SOD1 mutants. Among these, mitochondrial dysfunction appears to play a central role. Mitochondria quality control processes are key mechanisms evolved to ensure the maintenance of an adequate pool of functional mitochondria. This can be of vital importance in ALS mutant motor neurons, where there are extensive mitochondrial functional and structural abnormalities. Abnormal mitochondria are found in NSC34 motor neuron-like cells expressing mutant SOD1. We have investigated mitochondria quality control systems in NSC34 cells, stably transfected

with mitochondria-targeted (inter membrane space, IMS) and untargeted G93A-SOD1 constructs. We have measured markers of autophagy in relationship to mitochondria. We have detected increased levels of the autophagy adaptor p62 in NSC34 cells expressing G93A-SOD1, especially in cells expressing IMS-targeted protein. p62 co-localized with mitochondria, in both soma and neurites. Additionally, lysosomes, labeled with LysoTracker Red, accumulated in NSC34 differentiated cells expressing mutant SOD1. Studies of autophagy fluxes, determined in the presence of the lysosomal inhibitor bafilomycin A1 indicated a dysfunction in IMS-targeted G93A-SOD1 cells, but surprisingly not in untargeted G93A-SOD1 cells. The relevance of studying IMS-targeted G93A-SOD1 cells resides in the fact that mitophagy is determined exclusively by a mitochondrial damage, as the source of stress is by design confined to mitochondria. Further studies aimed at determining if the cellular pathways required to accomplish mitophagy are specifically impaired in SOD1 mutants or if the excessive accumulation of damaged mitochondria is due to clogging of the system will shed new light on the involvement of mitochondria quality control in ALS.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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E von Behring Chair for Neuromuscular and Neurodegenerative Disorders

Title: Elp3 is a disease modifier of amyotrophic lateral sclerosis

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Abstract: Amyotrophic Lateral Sclerosis (ALS), the most common motor neuron disorder of adulthood, is characterized by the degeneration of motor neurons in the spinal cord, brainstem and motor cortex resulting in muscle weakness, atrophy and spasticity. In a genome-wide association study, our group identified a polymorphism in the ELP3 gene to protect against ALS (1).This finding showed to have biological relevance as, within the same study, an independent genetic screen in *Drosophila* identified two different loss-of-function mutations in the fly homologue of ELP3 that induced aberrant axonal outgrowth and synaptic defects. Furthermore, lower expression levels of ELP3 were found in brain of individuals with the ALS at-risk genotype and finally the knock-down of Elp3 in the zebrafish induced motor axonal abnormalities similar to those induced by mutant SOD1 and mutant TDP43. Taken together, these initial findings suggested that low ELP3 expression may render motor neurons vulnerable to neurodegeneration and that overexpression may protect (1). ELP3 is the catalytic subunit of the Elongator complex, comprised of six subunits (ELP1-ELP6). Elongator is known to regulate translation efficiency - is involved in tRNA wobble modifications - and also transcription elongation - it associates with RNA polymerase II (2, 3). Little is known about proteins regulated or affected by ELP3, except for the stress response transcription factors Atf1 and Pcr1, which are downregulated in yeast lacking ELP3 after stress (4). We investigated the overexpression and knock-down of Elp3 in the SOD1G93A mouse model of ALS and also in the SOD1A4V zebrafish model. ELP3 overexpression was achieved both by intrathecal delivery of AAV9-ELP3 viral particles in neonatal mice and by generation of an inducible transgenic HuELP3 mouse. We found that Elp3 overexpression delays disease onset and prolongs survival of SOD1G93A mice whereas it deletion (Elp3 -/- mouse) leads to embryonic lethality at E9. We also found a protective effect of ELP3 on mutant SOD1-induced motor axonopathy in the zebrafish. Finally, we also investigated the molecular mechanism involved in Elp3 neuroprotective effect. We found that the neuroprotective effect of ELP3 in the SOD1A4V zebrafish is abrogated by mutations in the SAM domain, but not in the HAT domain. Further investigation is needed to clarify the role of Elp3 in neuroprotection in general and as ALS disease modifier in particular.
(1)Simpson CL et al. Hum Mol Genet. 2009 (2)Otero G, et al. Mol Cell. 1999 Jan;3(1):109-18. (3)Huang B, et al. RNA. 2005 Apr;11(4):424-36. (4)Fernández-Vázquez J, et al. PLoS Genet. 2013;9(7)

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Title: Identification of a resilient population of axotomized motoneurons within the mouse facial motor nucleus

Authors: *R. M. MEADOWS[1,2], M. M. HAULCOMB[1,2], T. BEAHRS[3], R. J. BATKA[1,2], N. D. SCHARTZ[1], V. M. SANDERS[4], K. J. JONES[1,2]
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Abstract: The rodent facial nerve axotomy model is a widely used injury paradigm to study motoneuron (MN) survival and regeneration. It is well-established that the majority of facial MN survive 4 weeks following facial nerve transection at the stylomastoid foramen in adult wild-type (WT) mice. In contrast, there is ~50% loss in facial MN at 4 weeks following facial nerve transection in immunodeficient mice, which can be effectively reversed with reconstitution of WT CD4+ T lymphocytes prior to axotomy. Thus, CD4+ T cells play a critical role in facial MN viability following facial nerve transection. Upon further investigation of this immune-mediated neuroprotection, our laboratory discovered that facial MN survival in both WT and reconstituted immunodeficient mice significantly decline to similar levels at 10 weeks post-axotomy. Therefore, the ability of the adaptive immune system to mediate facial MN survival after axotomy is transient, and appears to occur within a few weeks after injury. We hypothesize that two subpopulations exist within the mouse facial motor nucleus. One subpopulation, making up 50% of all facial MN, is dependent upon a functional peripheral immune system in order to survive from target disconnection to reconnection; and a second subpopulation that is capable of surviving for a prolonged period of time regardless of immune status or establishment of target reconnection. In order to test our hypothesis, we performed a facial nerve axotomy on WT and immunodefieinct mice and examined facial MN survival at 18 and 26 weeks post-axotomy. Our results indicate that the resilient subpopulation of facial MN persists indefinitely in WT and immunodeficient mice and remains at about 50%. We conclude that this resilient subpopulation of facial MN is viable regardless of immune status, and does not require target innervation, therefore presenting a unique opportunity for therapeutic intervention in peripheral nerve injury. We are currently exploring mechanisms underlying the distinct phenotypes.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Spinal Muscular Atrophy Foundation

Title: SMA motor neurons show impaired mRNP complex assembly

Authors: *P. G. DONLIN-ASP[1], C. FALLINI[1,4], J. P. ROUANET[1], M. E. MERRITT[1,2], G. J. BASSELL[1,3,2], W. ROSSOLL[1,2]
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Abstract: Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by a specific degeneration of motor neurons. SMA results from a reduction in the survival of motor neuron (SMN) protein, which is ubiquitously expressed with a well characterized role in promoting the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). While underlying defects in splicing have been observed in SMA models, these defects are not unique to motor neurons, leaving their role in the selective motor neuron degeneration unclear. Data from our lab and others led to the hypothesis that SMN plays a critical role in the assembly and/or trafficking of messenger ribonucleoproteins (mRNPs) in neuronal processes. Previously we have discovered specific defects in the axonal localization of mRNAs (*β-actin, Gap43*) and mRNA-binding proteins (HuD, IMP1) in axons and growth cones of primary motor neurons from SMA mice or depleted of SMN. We also observed that overexpression of both HuD and IMP1 can mitigate the effects of low SMN levels on axon outgrowth and GAP43 protein levels in growth cones. However, the molecular function(s) of SMN in the biology of mRNPs remained unclear due to

the lack of specific assays.To investigate the biological role of SMN and the effects of SMN deficiency in axonal mRNA regulation, we have established a trimolecular fluorescence complementation (TriFC) assay in motor neuron cultures as a sensor for mRNA and protein association, to validate the proposed role of SMN in mRNP assembly and localization. Our findings revealed a deficiency in the assembly of IMP1 protein/*β-actin* mRNA containing complexes in SMA motor neurons, which is consistent with SMN acting as a chaperone for mRNP complex assembly. These results are further supported by RNA immunopreciptation experiments from SMA mouse tissue extracts which show a substantial reduction in the levels of *β-actin* and *Gap43* mRNAs pulled down with IMP1. We can also show for the first time that SMN-deficiency causes defects in axonal local translation, likely a downstream consequence of impaired mRNP transport complex assembly.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 417.14/V3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Voxel-based mapping of grey matter volume and glucose metabolism profiles in Amyotrophic Lateral Sclerosis

Authors: *M.-S. BUHOUR[1], L. CARLUER[1,2], F. DOIDY[1], A. MONDOU[1,2], A. PÉLERIN[1,2], F. EUSTACHE[1], F. VIADER[1,2], B. DESGRANGES[1]
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Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease of the nervous system involving both upper and lower motor neurons. Automated whole-brain analysis techniques have been developed to quantify grey matter (GM) morphology using MRI T1-weighted images. Voxel-based morphometry (VBM) studies have yielded a number of discrepancies in their results. However, more and more studies have observed GM volume loss beyond motor and premotor cortices. Only two recent positron emission tomography (PET) studies, combined with 18F-fluorodeoxyglucose (FDG), investigated the effect of ALS on cerebral glucose metabolism, using a whole brain analysis method (Cistaro et al. 2012; Van

Laere et al. 2014). They reported both hypometabolism in bilateral premotor cortex and hypermetabolism in several posterior regions such as the medial temporal cortex and the cerebellum. The present study combined for the first time MRI and ^{18}FDG-PET to investigate morphological and functional brain abnormalities in a group of ALS patients. Method: Twenty-nine patients diagnosed with ALS according to the Revised El Escorial criteria (mean age 60.6 years old) and twenty-nine sex and age-matched normal volunteers (mean age 60.8 years old) underwent both ^{18}[F]FDG positron emission tomography and high-resolution 3 T MRI T1-weighted anatomical imaging. MRI data were handled using VBM5. The PET data were coregistered to their corresponding MRI, corrected for partial volume effect and normalized using the deformation parameters defined from the MRI procedure. A t-test was performed, using Statistical parametric mapping 5, with a threshold p(uncorrected)<0.001 with k>80 voxels. Results: compared with the healthy controls, ALS patients showed decreased GM volume mainly in left precentral gyrus as well as in several parts of the temporal, parietal and occipital lobes. We also found GM volume loss in subcortical regions such as the right amygdala, and the putamen and thalami on both sides. Hypometabolism concerned the left precentral and postcentral gyri and left inferior orbital part of the frontal lobe. Increased glucose metabolism consumption was also found in ALS patients in bilateral medial temporal lobe as well as in the cerebellum. Conclusion: ALS is not only associated with GM volume loss and hypometabolism in premotor and motor cortices as well as other cortical and subcortical areas, but also with hypermetabolism in the medial temporal cortex and the cerebellum. The mechanism of this phenomenon in ALS is still unknown and needs further consideration and research.

Disclosures: **M. Buhour:** None. **L. Carluer:** None. **F. Doidy:** None. **A. Mondou:** None. **A. Pélerin:** None. **F. Eustache:** None. **F. Viader:** None. **B. Desgranges:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NSO66888

Title: Micrornas at the *C. elegans* neuromuscular junction: Potential sma modifiers?

Authors: ***P. J. O'HERN**, A. C. HART
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Abstract: Spinal Muscular Atrophy (SMA) is a neurological disorder characterized by loss of lower motor neurons. This degeneration is almost exclusively caused by mutations in the SMN1 gene that lead to decreased levels of Survival of Motor Neuron (SMN) protein. smn-1 is the *C. elegans* ortholog of SMN1, disruption of which results in motor defects (Briese et al. 2009). It is unknown how loss of SMN protein perturbs motor neuron function, however evidence suggests that miRNA disruption may play a role (Mourelatos et al. 2003). miRNAs are small non-coding RNAs predicted to regulate protein expression of mRNAs. The RNA helicase Gemin3 is found in numerous complexes with SMN and also pulls down with numerous miRNAs in cultured mouse motor neurons (Dostie et al. 2003). I hypothesize that Gemin3-associated miRNAs are misregulated in smn-1 loss-of-function animals leading to NMJ defects. I have identified *C. elegans* orthologs of Gemin3-associated miRNAs that regulate neuromuscular junction (NMJ) signaling. smn-1 lf animals are defective on aldicarb and have reduced pumping, suggesting NMJ defects. Using tissue-specific rescue analysis and genetic epistasis, I will elucidate miRNAs that regulate NMJ function in the same pathway as SMN-1. I will also compile a list of potential mRNA targets for candidate miRNAs utilizing online bioinformatics tools, assigning priority to conserved targets with known synaptic function. For each potential mRNA target, I will confirm miRNA regulation and investigate whether expression of these targets is altered in smn-1 lf animals. These experiments will advance our knowledge of how SMN protein contributes to essential neuronal function and expand our understanding of how miRNA misregulation may contribute to neurodegeneration.

Disclosures: **P.J. O'Hern:** None. **A.C. Hart:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Health Research Board (RP/2007/283)

Science Foundation Ireland (08/INV.1/B1949)

Title: The role of bid in toll-like receptor signalling in glial cells following mutant SOD1-induced neuroinflammation in Amyotrophic Lateral Sclerosis

Authors: *S. KINSELLA, K. S. COUGHLAN, H. G. KOENIG, J. H. M. PREHN
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Abstract: Neuroinflammation is a pathological hallmark of neurodegenerative disease, with evidence of increased microgliosis and astrogliosis in Amyotrophic Lateral Sclerosis (ALS). Non-cell autonomous death of motoneurons mediated by activated microglia and astrocytes is proposed to be a key event in ALS aetiology. Toll-like Receptors (TLRs) are the master regulators of immune response and are highly expressed on glial cells, with elevated levels of TLRs -2 and -4 identified in the brain and spinal cord in ALS pathology. The Bcl2 family member, Bid has recently been shown to have a role in inflammatory regulation via interactions with the IKK component of the TLR-NF-κB pathway. This study examined both the role of Bid in the inflammasome and the effects of Superoxide Dismutase 1 (S0D1), mutations of which are associated with ALS, on TLR-induced glial activation *in vitro*. Transient transfection of microglia (BV-2) with $SOD1^{wt}$ and $SOD1^{G93A}$ revealed increased *tlr2*, *tlr4* mRNA and increased TLR2 and TLR4 membrane expression. Interestingly, the expression of the NF-κB target gene, COX-2, is decreased in BV2 cells following Bid siRNA transfection and subsequent treatment with motoneuron $SOD1^{G93A}$ -conditioned media, compared to control siRNA-transfected cells exposed to $SOD1^{G93A}$ -conditioned media. Furthermore, a decreased level of NF-κB activation in response to LPS stimulation was seen when Bid is inhibited in microglial (BV2) cells. Reduced CD45 and IL-1β expression is evident in the absence of Bid compared to *wt* microglia treated with either TLR2 (Pam_3CSK4) and TLR4 (LPS) agonists 24 hours post stimulation. Our data also shows a delayed IκBα degradation in $Bid^{-/-}$ primary microglia compared to *wt* upon LPS stimulation, indicating a differential role of Bid in the activation of NF-κB. This data demonstrates that TLR signalling is activated by overexpressed $SOD1^{wt}$ and $SOD1^{G93A}$, and that Bid may play an important role in mediating TLR-induced pro-inflammatory signalling in glial cells.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 417.17/V6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Axonal transport and translation of α- and γ-actin mRNAs are altered in Smn-deficient motoneurons

Authors: *M. MORADI, L. SAAL, R. BLUM, M. SENDTNER
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Abstract: Axonal mRNA translocation and local protein synthesis are crucial for axonal guidance, growth, maturation and maintenance of synapses in developing motoneurons. Therefore, impaired axonal mRNA trafficking and translation might contribute to the pathogenesis of various neurodegenerative diseases such as Spinal Muscular Atrophy (SMA). ß-actin mRNA and protein are highly abundant in the growth cone of developing motoneurons where they regulate cytoskeletal dynamics required for the axon outgrowth and path finding. Previous findings have revealed that the axonal localization and translation of ß-actin mRNA are disturbed in primary motoneurons of a mouse model of SMA. Interestingly, this observation correlates with reduced axon length and growth cone size suggesting that axonal ß-actin mRNA deficiency might be related to the SMA phenotype. In order to address whether axonal translocation and translation of α- and γ-actin mRNAs are also affected in Smn deficient motoneurons, we cultured motoneurons in compartmentalized microfluid chambers and analyzed these RNAs by qRT-PCR. We also investigated alterations in actin protein synthesis by transducing motoneurons with lentiviral eGFPmyr-act 3'UTR reporter constructs and quantifying newly synthetized proteins in the axon terminal using Fluorescence Recovery After Photobleaching (FRAP). Our data reveal that all three actin isoforms become translocated into axons of motoneurons, in contrast to sensory neurons. In addition, we found that α-actin is highly enriched in axons of motoneurons compared to ß- and γ-actin suggesting that this isoform might have a particular function during axon development. We also observed that actin is not only translated from ß-actin mRNA in motoneurons but also at significant levels from α- and γ-actin mRNAs. Upon Smn depletion, both axonal transport and translation of all three actin isoforms are impaired suggesting that dysregulation of axonal transport of actin is functionally associated with the SMA phenotype. Ongoing experiments will determine whether the different actin isoforms accomplish different functions in the axon development in motoneurons in comparison to sensory neurons and thus could explain why these cells are predominantly affected in spinal muscular atrophy.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: E-Rare/ANR Grant

Title: Dynein/dynactin mutations associated with amyotrophic lateral sclerosis and their effect on axonal transport and neuromuscular junction formation

Authors: *V. BERCIER[1,2,3], T. AUER[1,4], F. DEL BENE[1,2,3]
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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, which is mainly sporadic in nature. This progressive pathology has an estimated incidence of 1:1000 and generally leads to death within 2-5 years of diagnosis due to muscle wasting and severe motor neuron loss. Over the last years, mutations have been identified in both sporadic and familial ALS patients, interfering with the function of many genes, including DCTN1, which encodes for a subunit of the motor protein complex component dynactin. The dynactin complex serves as an adaptor for the dynein motor complex, responsible for retrograde axonal transport, and it is believed to regulate dynein activity and the binding capacity for cargos. Interestingly, axonal transport deficits have been reported in various neurodegenerative diseases owing to the fact that neurons are highly polarized cells that depend on active axonal transport for growth, establishment and maintenance of synapses. Defects in transport of material for development or clearance of detritus in the axon can lead to neuronal stress and cell death and could arise from different causes: preferential type of transport, varying load size, and depletion or dilution of the motor protein populations. In order to determine how retrograde axonal transport is involved in the pathogenesis of ALS, we are characterizing a mutant zebrafish line for dctn1 with regard to axonal development of primary motor neurons, formation and stability of the neuromuscular junction and the behavioral phenotype produced. Fast axonal transport defects are quantified in primary motor neurons using the GAL/UAS bipartite system and fusion protein tracking *in vivo* by confocal timelapse microscopy. We are investigating the transport dynamics of cargos such as endosomes, mitochondria, synaptic vesicles and neurotrophic receptors in the motor neurons of wild-type versus dctn1 mutant embryos *in vivo*, and over time. The yeast MSN/PP7 system allows us tagging and visualization of target mRNAs as they are transported to the synapse for local synthesis. As dynactin was reported to be essential to synapse stability, we are examining the formation and maintenance of the NMJ by immunohistochemistry (structure), by use of

synaptophysin-GCaMP for calcium imaging (function) and we will observe its integrity over time. Behavioral analysis of the mutant embryos will serve as readout of the defects at the motor neuron and NMJ level. We hope to elucidate key molecular mechanisms in ALS etiology by revealing the role of dynein in NMJ maintenance and identifying novel regulatory events in axon degeneration and muscle atrophy along disease progression.

Disclosures: V. Bercier: None. **T. Auer:** None. **F. Del Bene:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: TDP-43 associated proteins as modulators of TDP-43 aggregation and toxicity in primary neurons

Authors: C.-C. CHOU[1], O. ALEXEEVA[1], Y. ZHANG[1], B. MO[1], K. R. WILLIAMS[1], D. C. ZARNESCU[4], G. J. BASSELL[1,2,3], *W. ROSSOLL[1,2]
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Abstract: Among the growing number of known amyotrophic lateral sclerosis (ALS) disease proteins, TAR DNA binding protein 43 (TDP-43) has emerged as a key player. Nearly all sporadic and familial ALS cases are characterized by cytoplasmic aggregations of hyper-phosphorylated, ubiquitinated, and cleaved TDP-43 fragments and the loss of nuclear TDP-43. TDP-43 pathology is also common in frontotemporal dementia (FTD-TDP), as well as other TDP-43 proteinopathies. In rare cases, mutant TDP-43 can trigger the ALS disease process, establishing a clear causal role for TDP-43 in neurodegeneration. It is still not clear whether pathogenic aggregation of TDP-43 is causing a toxic gain of function that contributes to the neurodegeneration observed in patients. TDP-43 inclusion pathology may reflect an exaggeration

of normal accumulation of TDP-43 into cytoplasmic RNA granules under stress conditions. In this study, we investigated the effect of pathogenic TDP-43 and regulators of TDP-43 toxicity on mouse primary neurons. We identified distinct cytotoxic effects of full-length TDP-43 and its C-terminal fragment (TDP-CTF), and ALS-associated TDP-43 mutants (Q331K, M337V and A382T). As compared with the overexpression of full-length TDP-43, TDP-CTF and mutant TDP-43 had an increased toxic effect on primary neurons. In a yeast-two-hybrid screen we have identified TDP-43 interacting proteins, and further tested their effect on the accumulation of pathological TDP-43 aggregates. In our assays, overexpression or knock-down of poly(A) RNA-binding protein (PABP) modulates cell viability and the accumulation of TDP-CTF aggregates. In addition to our primary cultured mouse neurons, PABP mutations also modulate neurodegeneration in a *Drosophila* model of TDP-43 proteinopathy. We further show overexpression of full-length TDP-43 caused increased stress granule (SG) formation under conditions of oxidative stress. We found that TDP-43 was colocalized with TIAR, eIF3 and PABP in SGs, whereas, TDP-CTF reduced SG formation, and HuD and TIAR but not eIF3 were sequestered into TDP-CTF aggregates. Taken together, these results suggest that TDP-43-induced cellular dysfunction and resultant toxicity may be associated with an altered function of stress response pathways, contributing to the neuronal degeneration observed in ALS/FTD.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The effects of metabolic disturbances on the levels of misfolded SOD1 in ALS patient-derived fibroblast lines

Authors: *I. KESKIN[1], E. FORSGREN[1], J. GILTHORPE[1], E. TOKUDA[2], A. BIRVE[1], P. M. ANDERSEN[1], S. MARKLUND[2]
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Abstract: Introduction The neurotoxicity of mutant SOD1's is believed to be exerted by misfolded SOD1 species. The patho-mechanism is poorly understood but has been suggested to involve perturbation of mitochondria, induction of ER-stress, reduction of proteasome and

autophagy efficiency and aggregation. Another unresolved feature of ALS is why carriers of SOD1 mutations are apparently healthy until middle age, and then undergo rapid neurological decline. Perhaps age-related decline in proteostasis and energy metabolism contributes, amplified by vicious circles that increase the levels of misfolded SOD1 in the tissue. Objectives We have generated fibroblast lines from skin biopsies derived from 8 ALS patients carrying mutations in SOD1 (A4V, H46R, E78_R79insSI, N86S, D90A, L117V, D126fsX24, G127instggg); from 1 ALS and 1 FTD patient with C9orf72 GGGGCC-hexanucleotide repeat expansion, from 2 healthy control subjects. These cells, which express the mutant SOD1s under the native promoter, offer opportunities for exploration which are not accessible in the other model systems. We used the cells to gain information on the effects of various metabolic disturbances on the levels of misfolded SOD1. Methods We cultured the fibroblast cell lines under a variety of stress conditions and with/without inhibitors. Misfolded SOD1 from fibroblast extracts was measured with a specific ELISA (misELISA) as described (Zetterström et al. J Neurochem 2011;117:91). Results All fibroblast lines derived from the ALS patients contained more misfolded SOD1 than those from control individuals. The proteasome inhibitor bortezomib caused marked increases in misfolded SOD1 levels. Induction of ER stress with tunicamycin, inhibition of mitochondria with rotenone, and suppression of autophagy with 3-methyladenine decreased misfolded SOD1 levels. Hyperoxia might conceivably stabilize SOD1 by artificially promoting the C57-C146 disulfide bond. There were, however, no differences between cells cultured in physiological oxygen tension (4% O2) and hyperoxia (20% O2). Under none of the conditions could any detergent-resistant aggregates be demonstrated in the cell extracts. Conclusion Proteasome inhibition caused large increases in misfolded SOD1 levels. Other cellular perturbations occurring in ALS and aging did not per se induce increases in misfolded SOD1 levels.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Nemours Foundation

Title: Transcriptome profiling of spinal muscular atrophy motor neurons derived from mouse embryonic stem cells

Authors: *M. E. BUTCHBACH[1,4,2,7], M. MAEDA[1,4], A. W. HARRIS[1], B. F. KINGHAM[5], C. J. LUMPKIN[1,4], L. M. OPDENAKER[6], S. M. MCCAHAN[3,2,7], W. WANG[1,2]
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Abstract: Proximal spinal muscular atrophy (SMA) is an early onset, autosomal recessive motor neuron disease caused by loss of or mutation in SMN1 (survival motor neuron 1). Despite understanding the genetic basis underlying this disease, it is still not known why motor neurons (MNs) are selectively affected by the loss of the ubiquitously expressed SMN protein. Using a mouse embryonic stem cell (mESC) model for severe SMA, the RNA transcript profiles between control and severe SMA (SMN2+/+;mSmn-/-) mESC-derived MNs were compared in this study using massively parallel RNA sequencing (RNA-Seq). The MN differentiation efficiencies between control and severe SMA mESCs were similar. RNA-Seq analysis identified 3094 upregulated and 6964 downregulated transcripts in SMA mESC-derived MNs when compared against control cells. Pathway and network analysis of the differentially expressed RNA transcripts showed that pluripotency and cell proliferation transcripts were significantly increased in SMA MNs while transcripts related to neuronal development and activity were reduced. The differential expression of selected transcripts such as Crabp1, Crabp2 and Nkx2.2 was validated in a second mESC model for SMA as well as in the spinal cords of low copy SMN2 severe SMA mice. Furthermore, the levels of these selected transcripts were restored in high copy SMN2 rescue mouse spinal cords when compared against low copy SMN2 severe SMA mice. These findings suggest that SMN deficiency affects processes critical for normal development and maintenance of MNs.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Families of SMA

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SMA Europe

Title: A stem cell model of motor circuits 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: Neuronal circuit perturbations are emerging as important determinants in the pathogenesis of neurodegenerative diseases. Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by deficiency in the ubiquitously expressed SMN protein. SMA is characterized by loss of motor neurons (MNs), muscle atrophy and abnormal reflexes. In SMA mice, sensory-motor circuit dysfunction precedes motor neuron loss raising the possibility that SMN deficiency in premotor neurons may play a critical role in the pathophysiology of SMA. The effects of SMN deficiency in premotor neurons on MN survival and function are currently unknown. To address this, we developed a stem cell-based model of the motor circuit in which SMN protein levels can be selectively depleted either in both MNs and interneurons (INTs), or in only one of the two neuronal types. To do so, we employed MNs (Hb9::GFP) and INTs (Syn1::mCherry) differentiated from mouse embryonic stem (ES) cell lines with regulated RNAi knockdown of endogenous SMN. The survival of MNs was measured using whole-well automated fluorescence imaging. When MNs and INTs were co-plated, a time-course analysis revealed significantly reduced survival of SMN-deficient MNs but not INTs relative to controls. Purified MNs by FACS resulted in a similar extent of MN death induced by SMN deficiency, suggesting that MN loss is mediated via cell autonomous mechanisms. To study the effects of SMN-deficiency on motor neuron function, we employed intracellular patch clamp recordings. In co-cultures of SMN-deficient MNs and INTs we found an increase in MN membrane excitability compared to controls with normal SMN levels. MN output - measured by the

spontaneous firing frequency - was also significantly reduced by SMN deficiency. In contrast, FACS purified SMN-deficient MNs were not hyperexcitable. To address whether MN hyperexcitability and reduced output are governed by similar mechanisms, we studied the effects of SMN-deficiency in INTs when co-plated with normal MNs and vice versa. We found that the increased MN membrane excitability is due to SMN-deficiency in INTs only, suggesting non-cell autonomous mechanisms. However, the reduced spontaneous MN firing was a result of SMN-deficiency in both MNs and INTs, suggesting that this phenotype is governed by both cell autonomous and non-cell autonomous mechanisms. Collectively, our study reveals that dysfunction and loss of MNs are distinct events that occur downstream of SMN deficiency as a result of differential effects in specific neuronal types.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Institute of Neurological Disorders and Stroke Intramural Research Funds

Title: Characterization of iPSC derivatives from spinal and bulbar muscular atrophy patients

Authors: *I. KATS, C. GRUNSEICH, K. ZUKOSKY, L. GHOSH, G. HARMISON, L. C. BOTT, C. RINALDI, K.-L. CHEN, G. CHEN, M. BOEHM, K. H. FISCHBECK
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Abstract: Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an X-linked neuromuscular disease caused by a polyglutamine repeat expansion in the androgen receptor. The disease is known to cause degeneration of motor neurons through a ligand-dependent toxic gain of function, but the exact mechanism is not well understood. Motor neurons differentiated from SBMA patient derived induced pluripotent stem cells (iPSCs) provide a model for characterizing the disease mechanism and designing potential therapy. iPSCs were generated from six patients and compared to three control lines. Motor neurons were differentiated from the iPSCs and assessed for disease relevant phenotypes. The expanded polyglutamine repeats were unstable during re-programming, with several lines showing

expansion or contraction. Patient iPSCs expressed less androgen receptor than control lines but show androgen dependent stabilization and nuclear translocation with ligand exposure. Stem cell derived motor neurons showed immunoreactivity for HB9, Isl1, ChAT, and SMI-32, and were differentiated with similar efficiency between the patient and control lines, with no detectable difference appreciated following androgen treatment. Patient-derived motor neurons were found to have decreased HDAC6 levels and those with longer repeat lengths were shown to also have increased acetylated α-tubulin. This finding was confirmed in patient spinal cord sections and in stably transfected mouse cells. Another HDAC6-dependent process, intracellular lysosomal enrichment, was disrupted in motor neurons from iPSC lines with longer repeat lengths. The observed reduction in androgen expression, increase in acetylated α-tubulin and decrease in HDAC6 provide opportunities for further investigation of the SBMA disease mechanism and potential treatment.

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Poster

417. Motor Neuron Disease: Cellular Mechanisms

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ALSA

MDA

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MSCRF

Target ALS

Brain Science Institute

Title: Nuclear transport defect underlies C9ORF72 ALS/FTD neuronal injury in human neurons and is rescued by antisense and small molecules that target GGGGCC RNA

Authors: *C. J. DONNELLY[1,2], K. ZHANG[3], A. R. HAEUSLER[5], J. WANG[5], T. E. LLOYD[1,4], R. SATTLER[1,2], J. D. ROTHSTEIN[1,2,4]
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Abstract: A hexanucleotide repeat expansion in the C9ORF72 gene has recently been identified as the most common known genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Recent evidence employing iPS-neurons from C9ORF72 ALS patients suggests that the GGGGCC RNA products transcribed from the repeat expansion form complex G-quartet RNA structures and are neurotoxic. Similar to other repeat expansion disorders (e.g., myotonic dystrophy type I and II), the C9ORF72 GGGGCCexp RNA can sequester nuclear factors, including RNA binding proteins, and we hypothesize that these aberrant interactions are the primary cause of C9ORF72 neurotoxicity. Previous work from our laboratory has indicated that the GGGGCC RNA interacts with Ran-GAP1, a regulator of Ran-mediated cyto-nuclear trafficking. We have also found that Ran-GAP1 is a robust suppressor of neurotoxicity in a *Drosophila* model system that overexpresses GGGGCC RNA. Consistent with a Ran-GAP1 loss-of-function model, we have found that the G4C2 *Drosophila* model show reduced nuclear localization of NLS-containing reporters and iPS-neurons from C9ORF72 ALS patients exhibit perturbed Ran protein gradients. Importantly, these nuclear transport deficits can be rescued by treating the *Drosophila* and C9ORF72-patient derived iPS-neurons with antisense oligonucleotides that target GGGGCC repeat-containing RNAs or small molecules that bind G-quartet RNA structures to prevent any interaction with endogenous proteins. Taken together, these studies strongly support an RNA gain-of-function mechanism underlying C9ORF72 neurodegeneration. Moreover, we show, for the first time, that the toxic GGGGCCexp RNA reduces the nuclear import of classical NLS-containing proteins via an aberrant interaction with Ran-GAP1.

Disclosures: C.J. Donnelly: None. **K. Zhang:** None. **A.R. Haeusler:** None. **J. Wang:** None. **T.E. Lloyd:** None. **R. Sattler:** None. **J.D. Rothstein:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 417.25/V14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH-NINDS: R01NS078375

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The SMA Foundation

Families of SMA

Roche

SMA Europe

Title: Motor neuron hyperexcitability is induced by non-cell autonomous mechanisms in a mouse model of spinal muscular atrophy

Authors: *E. FLETCHER, C. SIMON, J. PAGIAZITIS, X. WANG, G. MENTIS
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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by reduced levels of the ubiquitously expressed SMN protein. The hallmarks of SMA are loss of motor neurons (MNs), muscle atrophy and abnormal postural reflexes. MNs innervating proximal muscles are more vulnerable than MNs innervating distal hindlimb muscles, resulting in a proximo-distal progression of the disease. The mechanisms leading to selective motor deficits are poorly understood. Using SMA mice, we have previously shown that sensory-motor circuit dysfunction precedes MN loss. Vulnerable MNs are abnormally hyperexcitable and exhibit reduced reflexes compared to resistant MNs at early stages of the disease. An indicator of MN dysfunction is an increase in intrinsic excitability. To address whether an increase in MN membrane excitability is caused non-cell autonomously by premotor synaptic dysfunction, or cell autonomously due to SMN deficiency per se in MNs, we studied the intrinsic membrane properties in wild type and SMA MNs using whole cell patch recordings from intact spinal cords *in vitro*. At pre-symptomatic stages, half of SMA MNs innervating proximal muscles were significantly hyperexcitable compared to their wild type counterparts. Their monosynaptically-evoked EPSPs after proprioceptive fibers stimulation revealed a significant reduction. In contrast, the remaining half of SMA MNs exhibited normal membrane properties and similar evoked EPSPs compared to wild type MNs. To test whether SMN deficiency in premotor neurons results in changes of MN membrane excitability, we studied the properties of MNs in SMA animals, in which SMN was selectively restored in only proprioceptive neurons. We used SMA mice harboring a Conditional Inversion allele which reverts SMN protein to normal levels upon Cre recombination. Parvalbumin-Cre mice were used to restore SMN protein levels in proprioceptive fibers. Our results reveal that the hyperexcitability observed in vulnerable SMA MNs is significantly corrected and restored the propioceptive-induced EPSPs. Restoration of SMN in MNs only, however, (using ChAT-Cre mice), did not result in any significant correction

in either MN excitability or synaptic function. These findings strongly suggest that SMN deficiency in proprioceptive neurons alters the intrinsic excitability of SMA MNs through non-cell autonomous mechanisms, consistent with SMA being a disease of motor circuits.

Disclosures: **E. Fletcher:** None. **C. Simon:** None. **J. Pagiazitis:** None. **X. Wang:** None. **G. Mentis:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Connecticut Stem Cell Research Grant 08-SCB-UCHC-022

UConn health center emergency grant

Title: Elucidating the degeneration of spinal motor neurons in human models of spinal muscular atrophy

Authors: ***C. XU**[1], K. DENTON[1], X.-J. LI[1,2]
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Abstract: Spinal muscular atrophy (SMA), the leading genetic cause of infant and toddler mortality, is caused by the decreased level of functional survival motor neuron (SMN) protein. How the deficiency of SMN, a universally expressed protein, leads to specific degeneration of spinal motor neurons has remained largely unknown. Here, we have generated induced pluripotent stem cells (iPSCs) from SMA patients using the episomal transduction method. Human iPSC lines from their relatives and normal individuals were also generated and utilized as controls. Spinal motor neurons were efficiently differentiated from these iPSCs by the application of both purmorphamine and retinoic acid. We then examined the mitochondrial fast axonal transport in motor neurons derived from SMA type I patient iPSCs and control iPSCs. Spinal motor neurons derived from SMA iPSCs displayed mitochondrial transport defects, including reduced motile mitochondria and a significant decrease in the frequency of retrograde events. SMA iPSC-derived spinal motor neurons were also specifically degenerated in long-term cultures, similar as what we observed in cultures derived from SMN-knockdown human

embryonic stem cells. Interestingly, N-acetylcysteine (NAC), a potent antioxidant, rescued the mitochondrial transport defects in SMA spinal motor neurons. The disease-related apoptosis and later motor neuron degeneration also ameliorated by NAC. These data suggest that impaired mitochondrial transport is involved in the motor neuron degeneration in SMA. Furthermore, we are using the transcription activator-like effector nucleases (TALENs)-mediated homologous recombination to restore the expression of SMN at different time windows to investigate how mitochondrial transport defects and motor neuron degeneration can be rescued. Together, we demonstrate the successful establishment of human pluripotent stem cell-based models of SMA and our models will be valuable for dissecting the pathogenic mechanism and screening compounds to rescue the motor neuron degeneration in SMA.

Disclosures: **C. Xu:** None. **K. Denton:** None. **X. Li:** None.

Poster

417. Motor Neuron Disease: Cellular Mechanisms

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Support: NIH Grant NS055925

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Salsbury Endowment

Sarepta Therapeutics

Title: Role of a unique RNA structure in regulation of alternative splicing of spinal muscular atrophy gene

Authors: N. N. SINGH[1], *R. N. SINGH[2]
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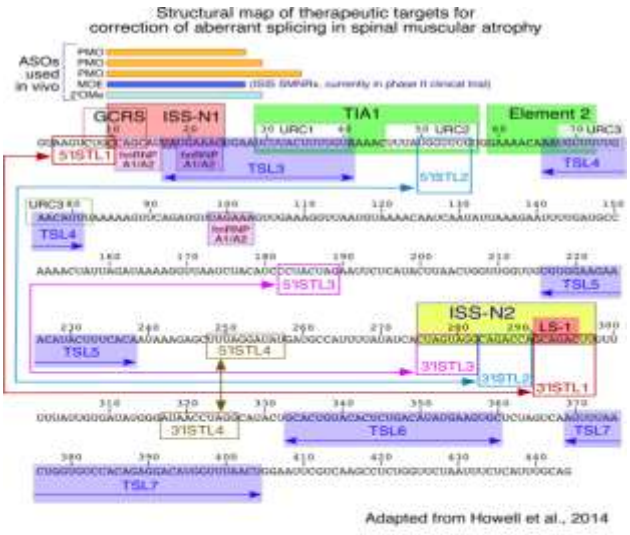
Abstract: Humans carry two copies of *Survival Motor Neuron* gene: *SMN1* and *SMN2*. Loss of *SMN1* coupled with skipping of *SMN2* exon 7 causes spinal muscular atrophy (SMA), a leading genetic disease associated with infant mortality. We have previously reported an intronic splicing

silencer (ISS-N1) as a negative regulator of *SMN2* exon 7 splicing (Singh et al., 2006). ISS-N1 is the most studied and highly promising target for an antisense oligonucleotide (ASO)-mediated splicing correction in SMA (Howell et al., 2014). ISS-N1 partially overlaps with a GC-rich sequence (GCRS) and also communicates with ISS-N2, another inhibitory element, which falls on a unique RNA structure that we term Internal Stem Through Long-distance interaction 1 (ISTL1) (Singh et al., 2013). Using site-specific mutations and SHAPE (Selective 2′-Hydroxyl Acylation analysed by Primer Extension), we show the formation and functional significance of ISTL1. Our results expand the repertoire of ASO-based targets for SMA therapy and underscore the therapeutic potential of regulatory information trapped in secondary and high-order RNA structures located within an intron. Our findings also demonstrate that an ASO-based approach could be employed to favourably remodel the structural map of intronic sequences that occupy a vast portion of human genome. **References:** Howell MD, Singh NN and Singh RN (2014) Advances in therapeutic development for spinal muscular atrophy. Future Medicinal Chemistry (In Press). Singh NK, Singh NN, Androphy EJ and Singh RN (2006) Splicing of a Critical Exon of Survival Motor Neuron genes is regulated by a human-specific silencer element located in the last intron. Molecular and Cellular Biology 26, 1333-1346. Singh NN, Lawler MN, Ottesen EW, Upreti D, Kaczynski JR and Singh RN (2013). An intronic structure enabled by a long-distance interaction serves as a novel target for splicing correction in spinal muscular atrophy. Nucleic Acids Research. 41, 8144-8165.



Disclosures: **N.N. Singh:** A. Employment/Salary (full or part-time):; Iowa State University, National Institutes of Health. B. Contracted Research/Research Grant (principal investigator for

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Poster

418. Down Syndrome Molecular Mechanisms

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: Fondecyt 1130241 (Chile, to P.C.)

National Institute for Aging grant 1 R21 AG041456-01A1 (to V. J.)

Title: Overexpression of the amyloid precursor protein triggers an increase in P21-activated kinase (PAK) activity and disrupts neurite extension in murine trisomy 16 neuronal cell lines, an animal model of Down syndrome

Authors: N. BARRAZA[1], A. CARDENAS[2], J.-V. BARNIER[3], K. S. POKSAY[4], V. JOHN[4,5], *P. A. CAVIEDES[1]
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Abstract: Down syndrome, or trisomy of autosome 21, is the chromosomal aneuploidy that most frequently survives pregnancy, and it is the most common cause of mental retardation of genetic origin Among the genes overexpressed from chromosome 21, several can potentially affect neural development and function. One particular gene is that encoding the amyloid precursor protein (APP). APP can bind to p21-activated kinases (PAKs) and affect their activities. Hence, APP overexpression can deregulate PAK activity and in turn disrupt several developmental and neural physiological processes. Although it has been described that APP binds PAKs through the APP C-terminal domain, it is not known if the complete membrane-bound APP or if its C-31

fragment are sufficient to activate PAK. C-31 is released by the action of caspases, which cleave APP at Asp664 in the C-terminus of APP. The increased activity of PAKs may alter actin dynamics and cofilin via phosphorylation of LIMK. This in turn affects the stability of actin filaments and reduces the number of dendritic spines, as well as axon growth, all of which constitute morphological features underlying cognitive impairment. Therefore, it is important to determine the interaction of APP with PAKs in a cellular model of Down syndrome, where APP is overexpressed, to determine how APP binds and activates PAK, and simultaneously how it can alter the stability of actin filaments and decrease neurite extension. In the present study, we utilized a neuronal cell line -named CTb - derived from the cerebral cortex of a trisomy 16 mouse (Ts16), an animal model of human Down syndrome, and a control cell line - named CNh- derived from the cortex of a normal littermate. We evaluated the interaction between PAKs and APP. APP overexpression was confirmed with immnoblotting and FACS techniques. Our results indicate that PAKs do not coimmunoprecipite with the 1-664 peptide derived from the caspase cleavage of APP, which lacks the C31 peptide, suggesting that this peptide is important for APP-PAK interaction. Our results also suggest that, under differentiating conditions, CTb cells exhibit fewer and shorter processes as compared to CNh cells. This work could lead to potential therapeutic targets to address the Down syndrome pathology.

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Poster

418. Down Syndrome Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 418.02/V18

Topic: C.06. Developmental Disorders

Title: Chromatin related functions of BRWD1, a neuronally enriched nucleosomal 'reader' protein: Implications for Down syndrome associated pathologies

Authors: *I. S. MAZE, W. WENDERSKI
Lab. of Chromatin Biol. and Epigenetics, The Rockefeller Univ., New York, NY

Abstract: Down syndrome (DS) is the most common chromosomal abnormality disorder in humans caused by a triplication of all or part of chromosome 21. DS is associated with physical growth delays, characteristic craniofacial abnormalities and intellectual disability. Although much is known regarding physiological aberrations associated with DS, far less is clear concerning the molecular mechanisms mediating this disease. Although DS is clearly marked by deficits in neural plasticity and increased neurodegeneration, few treatments exist that adequately reverse neuronal impairments resulting from this syndrome. Given that several neurological disorders associated with cognitive impairment result in disruptions in gene regulation, further mechanistic studies of neuronal specific histone function may provide clues into the underlying causes of neuropathology. Using SILAC in rodent neurons, coupled to LC-MS/MS, we identified H3K4me1, a classic genomic enhancer-enriched PTM, as the most abundantly enriched mark on dynamic nucleosomes. To understand the contribution of H3K4me1 to neurological function and plasticity, we set out to identify neuronally enriched reader/effector proteins binding to this mark *in vivo*. Synthesized N-terminal histone tail peptides were used to immunoprecipitate protein complexes associated with this mark from soluble nuclear extracts from brain; the chromatin effector protein Brwd1 was identified. BRWD1 is a WD40 repeat and bromodomain-containing protein that is encoded within the Down syndrome critical region 2 on chromosome 21 in humans. Given that WD40 domains have previously been shown to interact with other histone methylation marks, we sought to identify whether it might act as a multivalent binding protein that can recognize and simultaneously associate with multiple PTMs. Recombinant human BRWD1 WD40 and bromodomains were generated and purified, and custom-made histone PTM peptide arrays, as well as individual peptide IPs, confirmed these interactions *in vitro* with high affinity. Surprisingly, two other histone methylation signatures were identified as interacting partners for BRWD1, H3K27me3 and H4K20me3, both of which are transcriptionally repressive. Using a combination of biochemical and functional approaches, we are investigating the consequences of BRWD1 triplication in DS in relation to altered patterns of chromatin regulation in affected individuals, with the hope that such information may lead to the development of improved therapeutics aimed at alleviating DS associated pathologies.

Disclosures: I.S. Maze: None. **W. Wenderski:** None.

Poster

418. Down Syndrome Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 418.03/V19

Topic: C.06. Developmental Disorders

Title: DSCR1 is critical for local protein synthesis in neurons

Authors: *W. WANG[1], Z. SMILANSKY[2], K. CHANG[3], K. MIN[1]
[1]biology, The Ulsan Natl. Inst. of Sci. and Techno, Ulsan, Korea, Republic of; [2]2Anima Cell Metrology, Inc, Bernardville, NJ; [3]Zilkha Neurogenetic Inst. and Dept. of Cell and Neurobio., USC, Los Angeles, CA

Abstract: DSCR1 (Down syndrome critical region1, also called RCAN1 or regulator of calcineurin) is located on human chromosome 21 and highly expressed in the brain, especially enriched in hippocampal neurons. DSCR1 belongs to a conserved family of calcineurin (CaN) inhibitors called calcipressins, which includes RCN1P in yeast, CBP1 in fungus, nebula in *Drosophila*, as well as DSCR1 in mouse and human. The DSCR1 knock-out mice show learning deficits and impaired late-phase long term potentiation (L-LTP) which requires new gene expression, implying that DSCR1 may play a role in local protein synthesis. We discovered that DSCR1 plays a critical role in local protein synthesis by regulating FMRP (Fragile X Mental Retardation Protein) function in dendritic spines. Upon BDNF stimulation, DSCR1 becomes phosphorylated, which in turn changes DSCR1 from an inhibitor to an activator of Calcineurin. Active Calcineurin then dephosphorylates phosphorylated FMRP (pFMRP), leading to release of pFMRP from target mRNAs, thus allowing local mRNA translation. It is also known that local protein synthesis is critical for axon growth cone turning in developing axons. Here, we report that DSCR1 is present in axon growth cones and required for turning and development of axon growth cones. We show that DSCR1 is located in axon growth cones using SIM (Structured Illumination Microscopy) and STORM (Stochastic Optical Reconstruction Microscopy). Furthermore, using photoswitchable dendra 2 proteins and a new FRET technique, we show that DSCR1 is involved in local protein synthesis in axon growth cones. Together, our results imply that DSCR1 plays a critical role in local protein synthesis in dendritic spines as well as axon growth cones.

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Poster

418. Down Syndrome Molecular Mechanisms

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Program#/Poster#: 418.04/V20

Topic: C.06. Developmental Disorders

Support: Fondecyt 1130241

Title: The PAK effector pathway as a possible pathophysiological target in immortalized cell models of Down Syndrome. Possible role of DSCAM mediated dysregulation

Authors: *R. D. PÉREZ[1], J.-V. BARNIER[2], R. CAVIEDES[1], P. CAVIEDES[1]
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Abstract: Down syndrome (DS), or trisomy 21 in man, results from the presence of an extra copy of autosome 21, resulting in various multisystemic anomalies, where mental retardation is the most striking. The CTb cell line, derived from the cerebral cortex of a trisomy 16 fetal mouse (Ts16), an animal model of DS, is an model for the study of DS-related cell pathophysiological phenomena. When comparing CTb cells with the CNh cell line, a counterpart derived from the cerebral cortex of a normal animal, multiple DS-related gene impairments in neuronal function have been studied. One of such genes, which reportedly is involved in neuronal development and function, is dscam, which codes for the cell adhesion protein DSCAM (Down Syndrome Cell Adhesion Molecule). This protein recognizes Netrin at the membrane level, leading to a signaling cascade that is pivotal for correct neuritic development. In the latter case, the cellular signaling pathways involved are the serine/threonine p21-activated kinases (PAK), which then act downstream on effectors such as LIMK-cofilin, leading to reorganization of actin filaments. The present study shows that DSCAM is overexpressed in CTb cell by 40%, compared to CNh cells, as analyzed by western-blot and flow cytometry. This could increase PAK pathway function, in turn deregulating neuronal plasticity. This is supported by present results using Sholl analysis, which revealed a reduction in process number and length in CTb cells compared to CNh. A crucial question then arises, which involves verifying if PAK kinases are effectively deregulated in the trisomic condition. The present work further presents the correlation between dscam gene overexpression and its action on the PAK pathway, as determined by PAK activity phosphorylation states and morphological parameters of neuronal plasticity. The CTb cell line could be a model to assess relevant therapeutic in DS.

Disclosures: **R.D. Pérez:** None. **J. Barnier:** None. **R. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent protection. **P. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent protection.

Poster

418. Down Syndrome Molecular Mechanisms

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AG017617

AG014449

Alzheimer's Association IIRG-12-237253

Title: Effects of maternal choline supplementation (MCS) on CA1 pyramidal neuron gene expression in adult Ts65Dn and normal disomic (2N) offspring

Authors: *M. J. ALLDRED[1,4], S. H. LEE[2], E. PETKOVA[3,5], S. D. GINSBERG[1,4,6]
[1]Ctr. for Dementia Res., [2]Med. Physics, [3]Child Psychiatry, Nathan Kline Inst., Orangeburg, NY; [4]Psychiatry, [5]Child and Adolescent Psychiatry, [6]Physiol. & Neurosci., New York Univ. Langone Med. Ctr., New York, NY

Abstract: Down syndrome (DS) is the most frequent genetic cause of intellectual disability (ID) with an increasing prevalence in the USA, with current estimates of 1 in 691 live births. Individuals with DS have system-wide impairments, including the musculoskeletal system and notably the central nervous system, with ID and decreased cognitive function seen by impairments in hippocampal learning and memory, degeneration of cholinergic basal forebrain neurons (BFCNs), and language and communication skills. In addition to abnormal CNS function during development and adult life, individuals with DS develop Alzheimer's disease (AD) neuropathological hallmarks early in mid-life, including senile plaques (SPs), neurofibrillary tangles (NFTs), and early endosomal abnormalities. To examine the gene expression changes and test a putative treatment paradigm, we are using the Ts65Dn mouse model of DS/AD, specifically assessing CA1 pyramidal neurons. The Ts65Dn mouse model recapitulates several critical components of DS/AD, including cognitive dysfunction and BFCN degeneration, providing mechanistic assessments for translation to humans. We examined the Ts65Dn mice at the start of the BFCN degeneration and tested a maternal choline supplementation (MCS) paradigm, postulating that MCS will have a beneficial effect on the ID and septohippocampal degeneration seen in the Ts65Dn mice. We will test MCS treatment for amelioration of BFCN degeneration and track pathways that may enable understanding of the

underpinnings of cognitive decline and AD pathology. Preliminary microarray results on a custom-designed platform indicate that MCS produces significant gene expression level changes compared to age-matched unsupplemented maternal choline (UMC) offspring in CA1 pyramidal neurons independent of genotype. Specifically, alterations in several classes of transcripts including both glutamatergic and GABAergic neurotransmission, along with adenylate cyclases and AD-related genes were observed in both Ts65Dn and 2N mice. Preliminary genotype differences that are MCS sensitive include App, Bace1, Grik4, Grik5, GabrA3, and Gat2. Comparing MCS offspring to UMC offspring in Ts65Dn and 2N littermates will help to elucidate specific genes and signaling pathways that may be responsive to this early intervention. Moreover, these mechanistic studies will hopefully further our understanding of choline requirements for cognitive development. This approach also has translational viability as a low cost, non-invasive method of cognitive improvement for DS, as well as having a generalized neuroprotective effect that could delay onset and development of AD.

Disclosures: **M.J. Alldred:** None. **S.H. Lee:** None. **E. Petkova:** None. **S.D. Ginsberg:** None.

Poster

418. Down Syndrome Molecular Mechanisms

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Topic: C.06. Developmental Disorders

Support: NIH R01NS076503

USUHS APG-70-2479

Title: Age-dependent disruptions to signaling networks in the hippocampus of the Ts65dn mouse model of Down Syndrome

Authors: ***D. R. HOLMAN**[1], Z. GALDZICKI[1,2], X. XU[1], P. DAO[2], T. PRZYTYCKA[2]
[1]Anatomy, Physiology, and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]NIH, Bethesda, MD

Abstract: Down Syndrome is a progressively degenerative set of cognitive deficits, caused by triplication of chromosome 21, that affects 1 in 500 live births. The DS mouse model, Ts65Dn, has a triplicated segment of murine Chr. 16 that is homologous to human Chr. 21. A major phenotype of these mice is hippocampal-mediated learning and behavioral deficits and abnormal

hippocampal synaptic plasticity. Previous work from our lab in the hippocampus of the Ts65Dn mouse model of DS identified the upregulation of miR-155 and the abnormal expression of other miRNAs, the resulting downregulation of miR-155 and miR-214 targets. The bioinformatic analysis of the miRNA profile in Ts65Dn hippocampus revealed that signaling networks critical for neuronal proliferation, inflammation, and learning and memory are disrupted in the Ts65Dn hippocampus. Here, we apply RNA-Seq on hippocampal mRNAs isolated from two-week and two-month old Ts65Dn mice to identify the potential impact of these factors on the transcriptome at developmentally critical time point and during adulthood. We then use network and ontological analysis to identify candidate networks that are likely dysregulated, as well as to infer the well-investigated phenotypic outcomes in hippocampus of Ts65Dn mice and DS individuals. Our analysis confirms the dysregulation of networks previously identified (DYRK1A-NFATc, ERK, VEGF, PI3K/AKT). It also identifies previously unknown dysregulated pathways, such JNK, JAK/STAT, and IL6 responses.

Disclosures: D.R. Holman: None. **Z. Galdzicki:** None. **X. Xu:** None. **P. Dao:** None. **T. Przytycka:** None.

Poster

418. Down Syndrome Molecular Mechanisms

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Program#/Poster#: 418.07/V23

Topic: C.06. Developmental Disorders

Support: NIH Grant K08 NS069811

Title: Synaptic plasticity deficits in mouse models of Down syndrome and Alzheimer disease

Authors: *C. M. WILLIAM[1], L. SAQRAN[2], M. A. STERN[2], M. P. FROSCH[1], B. T. HYMAN[2]
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Abstract: Investigations using intact neural circuits in mouse models of Down syndrome (DS) may provide an approach to identifying the physiological basis for neurodevelopmental delay in the disease. Synaptic plasticity plays a critical role in the refinement and function of neural circuits and may be impaired in DS. To test this hypothesis, we have assayed visual system plasticity in a partial duplication mouse model of DS, the Ts65Dn line. Ocular dominance

plasticity (ODP) is the process by which loss of vision in one eye, monocular deprivation (MD), during a postnatal critical period, results in an increase in the area of primary visual cortex responsive to the non-deprived eye, in strengthening of responses to stimulation of the non-deprived eye and in weakening of responses to stimulation of the deprived eye. Using expression of the immediate early gene Arc to define the width of the domain of visual cortex responsive to non-deprived eye stimulation following a 4-6 day period of MD, we find that trisomic mice do not demonstrate the expansion in the coronal width of the responsive domain that occurs in similarly-treated, non-trisomic littermates (trisomic width, 944 ± 77.89 microns, n=5; non-trisomic width, 1087 ± 87.44 microns, n=13; p=0.0028, T-test). Optical imaging of intrinsic signals in awake mice was used to measure the magnitude of responses to stimulation of each eye before and after MD. Non-trisomic mice demonstrate strengthening of responses to non-deprived eye stimulation (pre-MD dF/F, 0.4%, n=5; post-MD, 0.57%, n=6; p=0.01), however, trisomic mice do not (pre-MD, 0.55%, n=9; post-MD, 0.46%, n=5; p=0.13). These data suggest that DS model mice exhibit defects in early postnatal developmental plasticity. Amyloid-beta accumulation secondary to amyloid precursor protein (APP) overexpression has been implicated in early-onset Alzheimer disease in DS, however, whether elevations in amyloid-beta can impair synaptic function earlier in life is less clear. We previously found that overexpression of mutant APP can block critical period ODP (William et al., 2012, J Neurosci 32(23):8004-8011). We find that transgenic mice that neuronally-express and secrete amyloid-beta in the absence of APP (BRI-Abeta40 and BRI-Abeta42 strains), also demonstrate critical period ODP deficits, measured by Arc assay and by optical imaging, suggesting that elevated expression of amyloid-beta alone can contribute to critical period ODP deficits. These studies suggest that synaptic plasticity defects may impair neural system function in DS and lay the foundation for future studies in model mice exploring the roles of duplicated genes, including APP, in causing synaptic dysfunction.

Disclosures: **C.M. William:** None. **L. Saqran:** None. **M.A. Stern:** None. **B.T. Hyman:** None. **M.P. Frosch:** None.

Poster

418. Down Syndrome Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 418.08/V24

Topic: C.06. Developmental Disorders

Support: Fondecyt 1130241

Title: Decreased intracellular traffic after induction of autophagy in the CTb cell line, derived from the cerebral cortex of a trisomy 16 mouse, an *in vitro* model of Down Syndrome

Authors: *C. F. ARRIAGADA, D. HERNÁNDEZ, R. PÉREZ, P. SALAZAR, P. CAVIEDES
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Abstract: Trisomy of the human chromosome 21 represents the most frequent cause of mental retardation in humans. The murine chromosome 16 presents a great homology with that human chromosome, and cell lines derived from mouse trisomy 16 (Ts16) represent an *in vitro* model of the human condition. In the present study, we evaluated the autophagyc flux in cell lines derived from the cerebral cortex of normal (CNh) and Ts16 (CTb) animals. Previous experiments have shown a decreased protein degradation ratio in the trysomic condition, and that there are no differences in structural and protease markers between CNh and CTb cell lines. To evaluate intracellular trafficking of autophagyc vacuoles, we transfected the cell lines with LC3-GFP, followed by autophagy induction. Degradative compartments were evidenced by red/DQ-BSA. In the CTb cell line, an exacerbated colocalization between LC3 and DQ-BSA was observed. pH-dependant GFP fluorescence emission could explain the minimal colocalization observed in CNh cell line. To evaluate this event, cells were transfected with a LC3-GFP/mCherry construct. After autophagy induction, colocalization was observed for an increased time lapse in CTb cell line. To evaluate if protease activity could decrease GFP emission, protease inhibitors were added. In such conditions, we observed no differences between the cell lines indicating that GFP fluorescence is pH-dependent and it is unrelated to protease activity. In flux experiments, we evaluated the presence of LC3-II by western blot. Our resutls show that alkalinizing agents such as ammonium chloride and bafilomycin A induced a delay in the accumulation of LC3II after autophagy induction in the CTb cell line. These results suggest the presence of a decreased vesicular traffic in the Ts16, which could explain some neuropathological phenomena related to intracellular accumulation events observed in individuals with Down Syndrome.

Disclosures: C.F. Arriagada: None. **D. Hernández:** None. **R. Pérez:** None. **P. Salazar:** None. **P. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent protection for the CNh and CTb lines.

Poster

418. Down Syndrome Molecular Mechanisms

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Topic: C.06. Developmental Disorders

Support: Baily Thomas Charitable Fund

Addenbrooke's Charitable Trust SD/9727

Title: Mitochondria are dysfcuntional in Down's syndrome

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Abstract: Down's syndrome (DS) is caused by the triplication of chromosome 21, which includes the gene encoding the amyloid precursor protein (APP). High risk of Alzheimer's disease (AD) and widespread AD neuropathology is characteristic of DS. However, although almost all people with DS show AD plaques and tangles, not all develop dementia, despite lifelong excess of amyloid beta (Aβ), suggesting involvement of other disease-modifying factors. Recent studies point to an etiological role for mtDNA mutations and mitochondrial dysfunction, which has been shown to predate symptoms of aging and affect brain development. Mitochondrial dysfunction is implicated in AD and mtDNA aberrations are an important cause of aging, which is precocious in DS and is the biggest risk factor for both DSAD and sporadic AD. Mitochondrial dysfunction warrants further investigation to elucidate the natural history of aging in DS. The current study was designed in two parts, *in vivo* and *in vitro*, ensuring a comprehensive assessment of DS mitochondria. Twenty eight DS participants and 24 controls took part in an *in vivo* phosphorous magnetic resonance spectroscopy (^{31}P-MRS) study assessing mitochondrial function in quadricep muscle through assessment of the recovery time of the phosphocreatine (PCr) pool after in-scanner exercise. DS participants showed a significantly longer recovery time of PCr (25.74 ±5.75 SD) compared to controls (20.8 ±4.6 SD; p=0.0034), indicating inefficient mitochondrial function. A significant relationship with age was found in both groups, (controls, p<0.05; DS, p<0.01). This cannot be accounted for by fitness as groups were matched for VO_2 peak. In addition, muscle biopsies taken from 18 DS adults were assessed using a gold standard histology technique to determine whether muscle fibres contain a mitochondrial deficiency, namely, cytochrome *c* oxidase-succinate dehydrogenase (COX-SDH) staining. COX is crucial to mitochondrial function and is affected by age-related accumulation of mtDNA mutations. COX negative fibres were counted and compared to age and sex matched controls. Findings from *in vivo* and *in vitro* data are considered together to determine the validity of each technique. We suggest that characteristics of DS including precocious aging, early dementia and sedentary lifestyle, could in part be accounted for by faulty energetics. Although people with DS have the most to gain from these studies, due to the certainty of development of AD pathology, DS is the best 'model' of understanding the early pathological changes of AD in the general population.

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Poster

418. Down Syndrome Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 418.10/V26

Topic: C.06. Developmental Disorders

Title: DYRK1A, a novel biomarker for Alzheimer's disease identified in plasma (AD) and LCLs (AD and DS)

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Abstract: Extensive brain amyloidosis, typically associated with dementia, has been noted at autopsy of older individuals who exhibited few or no cognitive complaints prior to death. Amyloid accumulates years before symptoms become apparent, and current tests depend on detection of either amyloid accumulation in brain or tau proteins in cerebrospinal fluid. Biomarkers that may help predict disease before onset or progression of symptoms are critically needed. We have previously shown in a peripheral organ that decreased expression of DYRK1A, a serine-threonine kinase active in brain and important for tau phosphorylation, induces NFkappa B activation and increased inflammation. Here, a two-hybrid screen for human DYRK1A extracellular interactants suggested the presence of DYRK1A in plasma. DYRK1A was subsequently detected by immunoblot in plasma from transgenic mouse models having different gene dosage of Dyrk1a and, consequently, different relative protein expression. We next measured plasma DYRK1A levels in individuals with Alzheimer's disease (AD) that present

either mild cognitive impairment (MCI-AD) or dementia relative to plasma from control subjects. DYRK1A but not DYRK1B levels were significantly lower in plasma ($p < 0.0001$) and in lymphoblastoid cell lines (LCLs) ($p < 0.05$) from AD patients as compared to age-matched controls. Further, plasma levels of several markers related to AD and to DYRK1A expression in mouse models (BDNF, ApoD, and homocysteine) were altered in this AD cohort. AD-like dementia is also common in older individuals with Down syndrome (DS) though with a much earlier onset. We analysed DYRK1A levels in LCLs from patients with DS: we found a decreased level of DYRK1A in patients with DS and dementia. These findings suggest that reduced DYRK1A expression might be a novel plasma biomarker for AD.

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Poster

418. Down Syndrome Molecular Mechanisms

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Topic: C.06. Developmental Disorders

Support: LuMind Foundation

Research Down Syndrome

NIH Grant 5R0138484-15

Title: Attenuation of SHH signaling due to trisomy 21

Authors: ***F. FERNANDEZ**[1], S. EDIE[2], N. ZAGHLOUL[3], D. KLINEDINST[1], J. LEBRON[1], N. KATSANIS[4], R. REEVES[2]
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Abstract: Animal models of Down syndrome (DS; human trisomy 21) exhibit attenuated responses to Sonic hedgehog (Shh) pathway activation, a deficit that might influence the presentation of several clinical phenotypes related to craniofacial and nervous system

development in people with the condition. In an effort to locate “causative” genes on chromosome 21 (Hsa21) that reduce hedgehog sensitivity when over-expressed, we built a library of 169 Hsa21 cDNAs, 149 of which have highly conserved murine orthologs. These cDNAs have been cloned into Gateway Entry vectors, which enable rapid subcloning into a flexible collection of Gateway Destination plasmids. For these experiments, we subcloned the clone set into the pCS2+ vector that allows transcripts to be made from a SP6 promoter and can also drive expression through a mammalian-specific promoter. We first screened for the effects of overexpression of each gene on *in vivo* readouts of hedgehog activity in zebrafish embryos where the developmental outcomes resulting from hedgehog down-regulation have been well described. For the zebrafish experiments, the clone-set was transcribed into mRNAs, injected into 1-2 cell stage embryos, and scored at five days post fertilization (dpf) for relevant phenotypes. In all, we found 10 'hits' that consistently produced visually-identifiable features associated with changes in signaling through the primary cilium and/or Sonic activity, including cyclopia, craniofacial compression, formation of distinct U-shaped somites, and heart edema. Subsequent experiments using combinatorial injections of positive candidates suggest that some of these genes act in additive fashion to increase the penetrance of these phenotypes. In a second program of study, we analyzed the effects of Hsa21 gene dosage on Shh pathway responses in Shh-LIGHT2 cells, an industry-standard *in vitro* system that has been traditionally used to uncover and optimize Shh agonists or antagonists. Plasmids encoding each Hsa21 gene were transfected with high efficiency in 96-well plates and Gli-luciferase activity upon SAG stimulation was measured alongside control transfection with a GFP Destination vector. About a dozen genes decreased LIGHT2 responses by circa 20% of baseline and some of these hits overlapped those characterized in the zebrafish screen. We are replicating these results and extending them to other assays of SHH response. Our preliminary data suggest that this approach will be useful to identify Shh modifier genes on Hsa21. These may represent druggable targets for intellectual disability and for treatment of Shh subgroup medulloblastoma in the typically developing population.

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Poster

418. Down Syndrome Molecular Mechanisms

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Topic: C.06. Developmental Disorders

Support: 5R01HD038384-15

LuMind Foundation

Research Down Syndrome

Title: Is a reduced cellular response to shh a "common denominator" for multiple phenotypes of Down syndrome?

Authors: T. DUTKA[1], N. SINGH[2], J. T. RICHTSMEIER[2], *R. H. REEVES[3]
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Abstract: Down Syndrome (DS) is caused by inheritance of three copies of human chromosome 21 (Hsa21). All individuals with DS display some level of craniofacial dysmorphology, brain structural and functional changes, and cognitive impairment. Previous work has demonstrated that Ts65Dn, a mouse genetic model of DS, recapitulates aspects of each of these defects. Developmental processes underlying midface skeletal hypoplasia and hypoplastic cerebellum have been linked to an altered Sonic Hedgehog (SHH) response in the Ts65Dn model, suggesting that an attenuated SHH could underlie many phenotypes observed in the Ts65Dn model and in DS. I.e., if all trisomic cells show a similarly reduced response to SHH, then up-regulation of the pathway in affected trisomic cells might ameliorate these (and other) phenotypes in multiple tissues. To investigate this hypothesis, Ts65Dn mice were crossed with *Ptch1*$^{tm1Mps/+}$ mice in which the canonical SHH pathway is up-regulated in every SHH-responsive cell during development due to the loss of function of one allele of the pathway suppressor, *Ptch1*. Ts65Dn; *Ptch1*$^{tm1Mps/+}$ mice were compared to Euploid; *Ptch1*$^{tm1Mps/+}$, to Euploid; *Ptch1*$^{+/+}$ and to Ts65Dn; *Ptch1*$^{+/+}$ for craniofacial, behavioral and brain phenotypes. We found that constitutive up-regulation of the SHH pathway in Ts65Dn did not normalize the craniofacial dysmorphology that arises due to trisomy. Further, haploinsufficiency for *Ptch1* affected some behaviors differently than trisomy and did not complement most trisomic behavioral deficits. However, a nest-building task in which Ts65Dn mice performed poorly was normalized to some degree in Ts65Dn; *Ptch1*$^{tm1Mps/+}$ mice. Finally, structural anomalies of the cerebellum were ameliorated in Ts65Dn; *Ptch1*$^{tm1Mps/+}$ mice, supporting previous observations of the central role of an altered SHH response in this trisomic phenotype.

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Poster

418. Down Syndrome Molecular Mechanisms

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Topic: C.06. Developmental Disorders

Support: KAKENHI Grant 25460077

Title: Comparative proteomic profiling reveals aberrant cell proliferation in the embryonic brain of Ts1Cje, a mouse model for Down syndrome

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Abstract: Ts1Cje, a mouse model for Down syndrome (DS) exhibits a number of the morphological, biochemical, and transcriptional changes seen in the human with DS. However, there are currently only few data on proteomic alterations in the brain of some mouse models for DS. To identify molecular candidates involved in the abnormalities of Ts1Cje mice, we here employed comparative proteomic analyses based on two-dimensional gel electrophoresis (2-DE). First, proteins from neonatal and postnatal (3-month-old) whole brains of wild-type (WT) and Ts1Cje mice were detected by staining with a fluorescence dye after 2-DE separation. No differences were detected in the proteins expressed in whole brain between WT and Ts1Cje mice (n=3 in each genotype). Although we also examined proteins from three brain regions, such as hippocampus, striatum and cerebellum, of WT and Ts1Cje mice at 3 months of age, no differences were detected between the genotypes (n=3 in each genotype). Whilst, five spots with differentially expression in the Ts1Cje brain were detected by 2-DE separation of brain proteins from WT and Ts1Cje embryos at embryonic day 14.5 (E14.5) (n=5 in each genotype). Identification of the five proteins in Ts1Cje embryos by peptide mass fingerprinting indicates that two proteins were classified to cell proliferation-related molecules, whereas other two proteins were associated to energy metabolism. Experiments focusing on cell proliferation demonstrated that the number of M-phase cells were detected immunohistochemically with anti-phosphohistone H3 antibodies, significantly increased in the ganglionic eminence (GE) of E14.5 Ts1Cje brain (n=3 in each genotype). Furthermore, *in vivo* BrdU labeling experiment revealed that cell proliferation in the GE was significantly increased in Ts1Cje mice compared with WT mice (n=3 in each genotype). Our findings suggest that the dysregulated expression of proteins

identified by a comparative proteomic analysis could participate in increased cell proliferation, which may associate with abnormalities in the DS brain during embryonic life.

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Poster

418. Down Syndrome Molecular Mechanisms

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Topic: B.07. Synaptic Transmission

Support: MRC Grant MR/J004049/1

Title: Functional analysis of novel collybistin missense mutations associated with intellectual disability

Authors: ***P. LONG**[1], P. WESCHE[1], V. M. JAMES[1,2], M. TOPF[2], K. HARVEY[1], R. J. HARVEY[1]
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Abstract: Selected $GABA_A$ receptor subtypes are clustered at inhibitory synapses via interactions with the scaffolding protein gephyrin, which in turn is targeted to inhibitory synapses by collybistin, a GDP-GTP exchange factor with neuroligin-dependent activity. Collybistin harbours three key functional domains: a N-terminal regulatory *src* homology 3 (SH3 domain), a RhoGEF domain (that binds gephyrin and activates the small GTPase Cdc42) and a phosphoinositide-binding pleckstrin homology (PH) domain that is vital for correct membrane apposition. Collybistin-mediated clustering of gephyrin does not appear to be dependent on Cdc42, since collybistin mutants lacking any detectable GEF activity are able to induce submembrane gephyrin clustering. Rather, collybistin binding to phosphatidylinositol 3-phosphate (PI3P), a phospholipid found in cell membranes, is pivotal since a PH domain mutant (R303N/R304N) interfering with phosphoinositide binding abolishes gephyrin recruitment to synaptic sites. We have previously shown that genetic defects in the human collybistin gene (*ARHGEF9*) give rise to a range of symptoms consistent with loss of several $GABA_AR$ subtypes, including anxiety, seizures and intellectual disability. Here, we report the structure-function analysis of new collybistin missense mutations in patients with intellectual disability, affecting amino acids in the RhoGEF and PH domains. All mutations resulted in defective collybistin-

mediated submembrane clustering of EGFP-gephyrin in recombinant systems. Mutant collybistin proteins co-localised with gephyrin in cytoplasmic aggregates. Although only one of the mutations identified (R356Q) is predicted to affect a PH domain residue required for binding to PI3P, pull-down assays from HEK293 cells transfected with pRK5-myc-collybistin revealed that all missense mutations investigated impaired binding to PI3P. Taken together, these results suggest that mutations in the either the RhoGEF or PH domains can impair binding of collybistin to PI3P, and that this is the most common pathomechanism for collybistin dysfunction in intellectual disability.

Disclosures: P. Long: None. **P. Wesche:** None. **V.M. James:** None. **M. Topf:** None. **K. Harvey:** None. **R.J. Harvey:** None.

Poster

419. Epilepsy: Networks

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: B.09. Network Interactions

Support: Wellcome Trust PhD studentship

Title: Divisive inhibition prevents abrupt transition from order to chaos in a neural field model

Authors: *C. PAPASAVVAS[1], Y. WANG[2], A. J. TREVELYAN[1], M. KAISER[1,2]
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Abstract: Experimental results suggest that there are two distinct mechanisms of inhibition in cortical networks: subtractive and divisive inhibition. Subtractive inhibition shifts the neuronal input-output function to the right without changing the slope, whereas divisive inhibition causes a reduction in slope or the maximal firing rate. Notably, recent experiments done using optogenetics show that these mechanisms are delivered by different populations of interneurons with a well understood connectivity between them and the pyramidal population. While most research has focussed on understanding the gain control, the role of these inhibitory mechanisms in regulating the dynamics of the network is less well understood. This work presents a novel mathematical model of this basic cortical circuitry, which incorporates the two inhibitory mechanisms. We investigated the role of these inhibitory mechanisms in terms of network dynamics, and particularly focussing on the transition from ordered to chaotic behaviour. We

show that the model incorporating divisive inhibition exhibits quite different behaviour compared to an equivalent model without divisive inhibition. The presence of divisive inhibition in the network prevents the abrupt transition from regular to chaotic dynamics across the parameter space. In contrast, in models which only have subtractive inhibitory elements, there are many cases where small changes in synaptic strength result in sudden flips from stable to chaotic network behaviour. Synaptic plasticity has been postulated as a mechanism by which the brain learns and stores information. Our results have interesting implications, therefore, for how such plastic changes impact on the stability of dynamic network activity patterns. It is also relevant to other network transition such as from physiological to pathological (epileptic) brain dynamics.

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Poster

419. Epilepsy: Networks

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Title: Variations in whole-brain functional connectivity across seizure chronification in a mouse model of mesial temporal lobe epilepsy

Authors: *J. M. CORTES[1,2], A. ERRAMUZPE[1], J. M. ENCINAS[2,3,4], A. SIERRA[2,3,4], M. MALETIC-SAVATIC[5], A. L. BREWSTER[5], A. E. ANDERSON[5], S. STRAMAGLIA[1,2,6]
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Bilbao, Spain; [3]Achucarro Basque Ctr. for Neurosci., Zamudio, Spain; [4]Univ. of the Basque Country (UPV/EHU), Leioa, Spain; [5]Neurolog. Res. Inst., Baylor Col. of Med., Houston, TX; [6]Dept. di Fisica, Universit degli Studi di Bari and INFN, Bari, Italy

Abstract: How does the brain become epileptic? Although this is a major question for modern Neurology, we do not have yet a proper answer. Despite of intense research in the field, epilepsy continues having today a high prevalence, still the only available treatments are merely palliative, and a large number of patients are pharmaco-resistant and require surgical removal of the epileptogenic area. To go further to the answer of this question, we propose here to address what are the changes in the pattern of functional connectivity (FC) occurring during the transformation from a healthy brain into an epileptic brain. To answer this question, we have analyzed longitudinal variations of FC patterns in the hippocampus in a mouse model of medial temporal lobe epilepsy (MTLE), induced by intra-hippocampal injection of kainic acid (KA), which resembles main features of human MTLE. Mice were implanted with bilateral intrahippocampal and intracortical electrodes immediately after the injection of KA, and discontinuous 4h video EEG recordings were obtained up to 42 days post injection. Data was converted to ASCII and postprocessed to calculate two different measures of FC: Network Synchronization Index (NSI), which is based on correlations, and the Interaction Information (II), a generalization of the mutual information to triplets to address the amount information (redundancy or synergy) bound up in the set of three variables. The two measures, NSI and II, were computed across the time-evolution of the disease and across different frequency bands. We found important differences in preictal and postictal states in comparison to the baseline pattern of inter-ictal activity, regarding the strength of synchronism and the amount of redundancy and synergetic interactions. In particular, we noticed that whilst the time-evolution of the FC pattern for low-frequency bands evolved with the severity of the disease, this did not happen for the time-evolution of synergy and redundancy, suggesting that the information character of the epileptogenic network was affected in a non-linear manner with the disease severity.

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Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: Dr. Ralph and Marian Falk Medical Research Trust

NIH Grant 1R01 NS08414201

Title: The relationship between paroxysmal depolarizations and high frequency oscillations in focal epilepsy

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Abstract: In the human neocortex, aberrant rhythmic bursts of neural activity between 80 and 150 Hz - high frequency oscillations (HFOs) - are suggested to be a hallmark of focal seizures and may aid in localization of the focus. Clinically, this activity is typically detected via EEG electrodes, which involves recording from a relatively large (cm sized) neuronal network. However it is not clear whether such a large area is required for HFO generation or what mechanisms underlie its development. We hypothesize that HFO activity during seizures is (1) generated by microscopic networks, in association with paroxysmal depolarizing shifts (PDSs) of neurons, yet (2) remains detectible in macroscopic recordings because of: (a) volume conduction (a linear process obeying the rule of superposition) and (b) synchronization of many local networks during a seizure. We determined the presence of HFOs in single cell activity (intracellular recordings) and microscopic networks (extracellular recordings) from slice recordings of human neocortex during experimental seizures, characterized by series of PDSs. Using the multi-taper spectral estimate to reduce leakage across frequency bands (Thomson, 1982), we determined that power in the 80-150 Hz band was overtly present in single cell and sub-mm network recordings. This observation supports part (1) of our hypothesis that small networks are capable of generating HFOs. Part (2) of our hypothesis was examined using two approaches: processing of the human slice data and the use of multi-electrode array (MEA) recordings from epilepsy patients. Averaging intracellular and extracellular slice measurements was used to mimic volume conduction. Two types of averages were computed: one type triggered by PDS bursts (synchronous case) and the other used randomly selected epochs (asynchronous case). We detected significantly more HFO activity in the synchronous case as compared to the asynchronous case ($p<0.01$), supporting our hypothesis that volume conduction of synchronized neural activity can generate compound signals that include HFOs. Using recordings obtained from MEAs implanted in patients with epilepsy, we also detected HFOs during clinical seizures. Spectral estimation of both individual microelectrodes as well as averaged activity across the array (approximating clinical macroelectrode recordings) indicate

there is an increase in high gamma rhythmic activity within the focal seizure core when seizures are fully developed (Weiss et al, 2013). Our data suggests a small-network source for clinically detected HFOs and may be used for improved localization of the seizure focus.

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Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

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Epilepsy Research UK Grant

Medical Research Council

Title: Widefield imaging of sensory and epileptiform activity in mouse visual cortex

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Abstract: During epileptic seizures, cortical activity spreads in a pathological and escalating manner. Does this spreading activity bear any resemblance to the flow of activity seen during normal processing? Moreover, does it depend on lateral connectivity, and therefore on the functional architecture of the underlying cortex? To image cortical sensory responses and epileptiform discharges we expressed GCaMP3 in excitatory neurons with a Emx1-GCaMP3 line. Mice were implanted with a cranial window to image the visual cortex. A hole in the coverslip allowed injection of a chemoconvulsant and insertion of a pipette to record the local field potential (LFP). All measurements were made in awake mice. We first used widefield imaging to measure visually-evoked activity and map the retinotopic organization of visual cortex. Stimuli were flickering gratings (2 Hz) at various eccentricities (0-135 deg). GCaMP3 fluorescence reported neural responses oscillating at twice the stimulus frequency. The amplitude of these oscillations (0.13±0.01 %dF/F) yielded high-quality maps of retinotopy in primary visual cortex (V1) and higher visual areas; their phase indicated a wave of activation travelling

away from the stimulated region in V1 and a delayed activation of higher visual areas. We then induced an epileptic focus by injection of pilocarpine (5 M, 300nl) into layer 5 of V1. LFP recordings showed an escalation of epileptiform activity. Within minutes sharp LFP waves (i.e interictal spikes, IIS) increased in size (1.1±0.1mV) and frequency (0.42±0.03 Hz) and gradually evolved into complex-spike trains and ictal-like events. This activity persisted for ~30 minutes and then gradually declined. GCaMP3 reliably reported epileptiform activity, yielding large signals that dwarfed those seen during normal visual processing (38±3 %dF/F). To study the dynamics of this activity we used a standing-wave model, the product of a fixed time course and a fixed footprint in space. This model provided a good fit for the IIS-triggered averaged calcium variations (explaining 53% of the variance), revealing a standing wave of activation around the epileptic focus. However, ictal-like events displayed more complex dynamics, possibly resembling travelling waves. These results indicate that widefield imaging of GCaMP3 fluorescence is a powerful technique to study normal and epileptiform cortical activity at a mesoscopic scale. This technique is revealing intriguing similarities and differences between visual response propagation and epileptiform dynamics. Our current experiments aim to determine the relationship between these dynamics and the functional organization of the visual cortex.

Disclosures: **L. Rossi:** None. **D.M. Kullmann:** None. **M. Carandini:** None. **R. Wykes:** None.

Poster

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Support: FWO Research Project grant G074509N

PFV/10/008

Title: Effective connectivity of the macaque amygdala after kindling assessed by electrical microstimulation during functional MRI

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Abstract: Amygdala kindling is a widely used animal model to study network changes during mesial temporal lobe epileptogenesis. The aim of the current study was to study the effective connectivity in fully kindled macaques using electrical microstimulation during fMRI. Two rhesus monkeys were implanted with low impedance electrodes in the right amygdala. Electrical kindling consisted of daily stimulation with a 1 second, 60Hz sine wave at the intensity of the afterdischarge threshold (500μA monkey S, 1100μA monkey K). Monkeys were considered fully kindled when they showed stable stage 4 seizures (bilateral tonic-clonic movements), which was after 515 days of electrical kindling in monkey S and 477 days in monkey K. When fully kindled, the monkeys were injected with a contrast agent and scanned at a 3T Siemens MR scanner with an 8-channel phased-array coil under ketamine/medetomidine sedation. The fMRI protocol consisted of interleaved stimulation and no-stimulation blocks. During stimulation blocks, the amygdala was stimulated with a squared bipolar pulse (pulse width 0.48ms, frequency 200Hz, duration 250ms, intensity 800-1000μA) every 2500ms. No seizures were evoked during fMRI microstimulation. Electrical microstimulation of the kindling site in the amygdala during fMRI revealed extensive fMRI activations in cortical and subcortical structures in both monkeys. In monkey S, we observed strong significant activations in the ipsilateral putamen, thalamus, somatosensory cortex, early visual areas and in the contralateral cerebellum. In monkey K, the main activations were observed in the contralateral amygdala, claustrum, thalamus, superior temporal sulcus and the cerebellum. A conjunction analysis of the two monkeys (at a threshold of $p<0.01$ uncorrected for each monkey) revealed common fMRI activations in the contralateral amygdala, pallidum, thalamus and cerebellum. Therefore, the amygdala in stage 4 kindled monkeys is connected to extensive, bilateral networks of cortical and subcortical structures. These networks were distinct but partially overlapping in the two animals.

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Poster

419. Epilepsy: Networks

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Program#/Poster#: 419.06/W4

Topic: B.09. Network Interactions

Title: Grouping inter-ictal and pre-ictal epileptic states through a complex networks approach

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Abstract: Complex networks analysis has allowed the study of brain connectivity in anatomical and functional contexts. In the pathological brain, the study of complex networks has revealed abnormal interactions between different areas that can be associated with a strong hub-like region tending to produce abrupt synchronizations in the brain as is the case in epilepsy. Despite this, it is still not well known if the analysis of complex networks in epilepsy can be useful in the identification of periods with increased risk of epileptic seizures. For this, we constructed and analyzed networks based on intracranial EEG recordings from epileptic patients (n=17) presenting focal and pharmacological resistant seizures. In particular, we selected a total of 87 seizures having at least 8 hours of preceding inter-ictal periods (seizure-free periods) for a total of 715 analyzed hours and 1457 electrodes. For each seizure, we estimated different features within consecutive, non-overlapping windows of 3 seconds along the whole preceding onset period. All features were related to activities in frequency bands varying from 0.3 to 420 Hz. For each of these bands, we constructed adjacency matrices between all pairs of available electrodes based on the absolute value of the normalized cross-correlation taken from the corresponding pair of frequency activities inside consecutive 1-minute windows. We subsequently defined a connectivity threshold of 0.75 above which we established an existing connection (link) between a particular pair of electrodes (nodes in the network). Based on this, we dynamically constructed networks for which we estimated six complex measurements including clustering index, density, and characteristic path length among others. We then implemented a clustering algorithm (k-means) to observe if epileptic activities could be grouped into inter-ictal and pre-ictal (ictal-facilitating) periods, according to a defined grouping criterion. We report that for 12 patients (71 %), a minimum of one pre-ictal cluster could be identified in at least one of the analyzed seizures with an average of 2.75 out of 5.25 seizures per patient and a total of 33 seizures out from 63 (52 %). Furthermore, pre-ictal clusters were found to start between 20 and 102 minutes (average 38 min) before seizure onsets. In general, qualitative observations showed that most of the pre-ictal clusters were related to a decreased connectivity between nodes, though for a few number of seizures the opposite was also observed. In conclusion, our results show that the analysis of complex networks could offer new and useful information for the identification of seizure facilitating periods.

Disclosures: K. Guarin: None. **M. Valderrama:** None. **M. Le Van Quyen:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Title: Hidden patterns might reveal new synchronies in biological distributed information networks: Examples from human intracerebral recordings and artificial data

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Abstract: The concept of binding by synchrony has been proposed as a mechanism to explain how information about a given stimulus is processed in a distributed network of neurons. If defining the boundaries of a physiological network is important, this identification in epilepsy is crucial for treatment. In 10-15% of all epileptic patients, the identification of this network requires intracranial electrodes. For a long time, emphasis has been put on the identification of low voltage fast discharges, which could be a specific signature of the epileptogenic tissue. Even though network periodicities have been related either to physiological or pathological states, recent data do not fully confirm such direct correlations. The likelihood quantification of regions involved in the seizure was introduced with the 'epileptogenicity index' (EI). The EI depends on the frequency spectrum and is defined as the ratio between high and low oscillations in a given time step. This practical approach assumes that two areas are involved in a seizure when their signals are similar in the frequency domain. Tackling this problem otherwise, we considered that two interacting processes must exchange data, for instance by copying parts for further processing. If true, synchronization could be detected by tracing electric field signatures. To solve this question we developed a codification that exposes only the signal shape. We then adapted a method used for data compression, the Context-Tree Weighting (CTW) algorithm, to search our stream for repeated patterns. Then we generated EEG-like signals fading into one another. The CTW was able to determine the coefficients used to mix signals better than the EI. Finally we studied data taken from deep electrodes of an epileptic patient, showing that both CTW and Granger's causality, in a similar but not identical fashion, highlighted correlations that the EI did not reveal (Fig. 1). These results suggest that complementing the frequency analysis with the recognition of shared electric field patterns might reveal relations that could help investigate neurological networks.

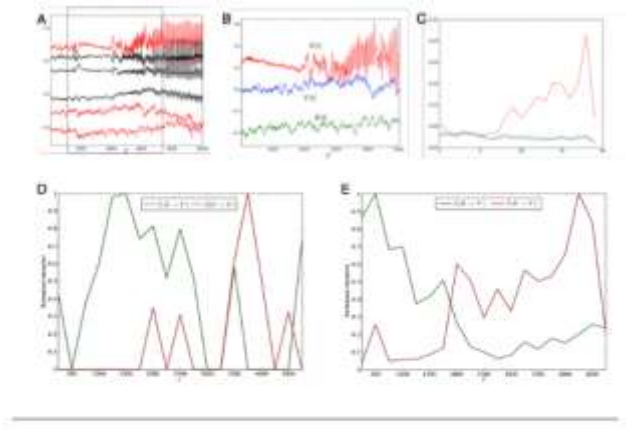

Fig. 1. Example of emergent and vanishing signal correlations that were not revealed by the EI index. (A) We first selected three channels (plotted in red), the first is clearly epileptogenic while the third is not; the second presents mixed features. (B) Plot of the selected channels, S(t) the seizure one (red), B(t) the basal one (green), Y(t) the probed one (blue). (C) EI index for each channel under study. (D) Normalized influence of B(t) into Y(t) (green) and S(t) into Y(t) (red) using the CTW estimator with a quantization of the EEG signals in 2 levels. (E) Normalized influence of B(t) into Y(t) (green) and S(t) into Y(t) (red) using Granger's causality estimator. From time step 2000 one observes correlations between {B(t), S(t)} and Y(t) that were not revealed by the EI index.

Disclosures: **A. Principe:** None. **A. Tauste-Campo:** None. **G. Deco:** None. **R. Rocamora-Zuñiga:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: Texas Instruments

Title: Identifying the epileptogenic zone using directed information

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Abstract: The 'epileptogenic zone' (EZ) refers to the core brain region responsible for the generation of seizures in patients with focal epilepsy. Visual analysis (VA) of the electroencephalogram either recorded from the scalp or from intracranial electrodes (electrocorticogram, ECoG) remains the gold standard for diagnosing the EZ, despite its

subjective and time-consuming nature. In this work we report pilot results on a novel quantitative technique, directed information, to identify the EZ from the ECoG recordings in a patient with focal epilepsy undergoing intracranial electrode evaluation. Directed information, an information theoretic quantity, quantifies Granger causality, which is itself a measure of effective connectivity - the network of causal interactions between distinct units in the brain. In this work, the units correspond to the different ECoG electrode contacts. The main contribution of this work is broadening the notion of directed information to continuous-valued random processes like ECoG, and then use it to estimate the effective connectivity to identify the EZ. The results are compared with conventional VA.

Disclosures: **R. Malladi:** Other; ECE, Rice University. **G.P. Kalamangalam:** None. **N. Tandon:** None. **B. Aazhang:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: NIH Grant R21 NS078301

Graduate Fellowship Lily's Fund

Title: The spatiotemporal characteristics of epileptiform activity in the dentate gyrus

Authors: *B. J. WRIGHT[1], M. JACKSON[2]
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Abstract: The dentate gyrus (DG) is thought to act as a filter or gate for signal entry into the trisynaptic circuit of the hippocampus. When this gating function is compromised, abnormally high electrical activity can spread through the hippocampus and generalize to other brain regions to initiate seizures. The dentate gyrus contains a powerful circuit of recurrent excitation formed by excitatory reciprocal synapses between granule cells and hilar mossy cells that can contribute to the processing of cortical inputs. The GC-MC-GC circuit has an architecture that may be capable of sustaining reverberations and generating abnormal electrical activity. To explore the role of dentate gyrus circuitry in seizure activity we used voltage imaging with voltage sensitive absorbance dye (RH 482) to investigate the spatiotemporal characteristics of epileptiform

activity in rat hippocampal slices. Slices were subjected to repetitive stimulation of the granule cell layer (20 Hz) in a $GABA_A$ receptor inhibitor and 5 mM KCl, to transform them to a susceptible state. Optical signals showed larger areas and delayed repolarization, compared to control responses recorded prior to induction. Results from thresholding experiments in which electrical stimulation was applied to the GC-MC-GC pathway revealed the minimum current at which epileptiform activity could be evoked. Electrical stimulation evoked epileptiform activity that was widely distributed throughout the slice; and there were no differences in the hyperactivity in the upper and lower blades. Epileptiform activity spread radially from the site of stimulation, and propagated to both blades of the DG before transmission to the CA3 region. Supported by R21 NS078301 and a graduate fellowship from Lily's Fund.

Disclosures: B.J. Wright: None. **M. Jackson:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: Swedish Research Council

Title: *In vivo* low intensity optogenetic train stimulation in the mouse hippocampus produces epileptiform afterdischarges

Authors: *F. BERGLIND, M. KOKAIA
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Abstract: Electrical stimulation in brain structures like the hippocampus has long been used to produce seizures in rodent epilepsy models. However, because of the non-specific nature of electrical stimulation and the recording artifacts inherent to its use, underlying network mechanisms involved in the generation of self-sustained activity (afterdischarges, ADs) can not be effectively dissected. With optogenetic techniques, discrete stimulation of specific neuronal populations is now possible and, combined with local field potential (LFP) recordings, we have utilized this to generate and record ADs in the mouse hippocampus *in vivo*, in a location and time dependent manner. It is also possible to follow the immediate dynamics of the induced LFP during the stimulation itself. We can show that ADs are more effectively generated when stimulating the cell bodies of the CA3 pyramidal layer rather than processes in the radial or

oriens layers, or in the CA1 or hilus. Additionally, at the low light intensity used in our studies (recruiting only a small proportion of hippocampal neurons) ADs only appeared after repeated stimulations. To achieve maximal amplitude and duration, recruitment of the contralateral hippocampus was required. The results of the present work contribute to better understanding the network involved in seizure induction.

Disclosures: F. Berglind: None. **M. Kokaia:** None.

Poster

419. Epilepsy: Networks

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NIH 086364

Title: Stochastic determinants of transitions to and from seizure states in a computational network model

Authors: *W. B. SWIERCZ[1,2], K. P. LILLIS[1,2], K. J. STALEY[1,2]
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Abstract: We used a computational model of hippocampal area CA3 to study ictogenesis in post-traumatic epilepsy. The model contains 22374 of integrate-and-fire neurons. They are arranged into two layers of 105 x 105 pyramidal cells and one layer of 18x 18 interneurons with GABAergic outputs. Connections between neurons were stochastically generated accounting for distance between cells and for the neuron type. Traumatic cell loss was equally and randomly distributed between both pyramidal cells and interneurons by removing subset of cells and corresponding inputs and outputs from the network. The result was a strong decrease in the level of spontaneous activity. Recovery after injury was modeled by recurrent axon sprouting and synaptogenesis. New connections between surviving cells were randomly generated using various strategies. Axon sprouting continued until surviving cells reconstituted the original number of synapses onto and from each neuron. The outcome of this recovery process varied depending on the sprouting strategy. For some networks, spontaneous activity returned to the

control level while many networks developed bursts of synchronous activity that increased in frequency and in fraction of participating neurons. Some networks developed seizures comprised of sustained (tonic) ictal-like activity that slowly transitioned into intermittent (clonic) activity. Recurrent sprouting was necessary but not sufficient for ictal-like activity. In networks with identical connectivity the transition from bursting to ictal activity depended on the initial variance, but not the mean, level of activity-dependent depression at individual glutamatergic synapses. Seizure termination occurred as a spontaneous transition from tonic to clonic activity and eventually back to periodic synchronous bursting. These transitions were not the result of global activity-dependent synaptic depression, but depended instead on changes in the network-wide variance of synaptic depression. Here we dissect the synapses where the variation in synaptic depression was most critical. These synapses on a small subset of cells and corresponding connections are responsible for dramatic changes in the mode of activity of the whole network. We are currently testing the hypothesis that ictogenesis can be disrupted in-vivo by modifying or disabling the synaptic connectivity of the most active cells in the network only. The negative impact on network performance may not be critical, although it may be technically very hard to locate and target this relatively small subset of cells.

Disclosures: W.B. Swiercz: None. **K.P. Lillis:** None. **K.J. Staley:** None.

Poster

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Title: Transition mechanisms from nonsynaptic epileptiform activity to spreading depression

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Abstract: Introduction: Invariably, when nonsynaptic epileptiform activity (NEA) induced in the dentate gyrus of hippocampus slices (HS) are recorded, the transition to spreading depression (SD) can be seen. During this transition, the interplay of the different ionic species involved and

the dynamic mechanisms of action responsible for the ionic homeostasis are too complex to be experimentally accessed. Thus, we investigated the mechanisms responsible for this transition through the correlation between experimental findings and computational simulations. Experimental recordings: NEAs were experimentally induced in rat HS in interface chamber. When the slices were perfused with high-potassium (10mM) and zero-added calcium, the NEAs appeared spontaneously after 1h. The extracellular potential was recorded in the granule layer. Computational model: This model describes the electrodiffusion processes of the ionic current through the neuronal and glial membranes and along the extracellular space (ES). The mechanisms incorporated to simulate the ionic homeostasis were: Na+/K+ pumps, Cl- cotransporters, Na+/H+ exchangers and chloride/carbonate exchangers. Cell volume changes were also calculated. The ephaptic effect, the gap-junctions and the ionic fluctuations were the non-synaptic connections between neurons. The transmembrane potentials were calculated in dependence on the ionic concentrations. Network was formed by functional unities composed by granule cell body, glial segments and the ES, configured in a 50x9x9 matrix. The model was implemented in Fortran. Results: The experimental findings showed NEAs blockage by tetrodotoxin (TTX), indicating the involvement of the Na-voltage dependent channels (NVDC) in the NEAs onset. After transition to SD, the events were also blocked by TTX. Simulations showed that the NEAs onset of each event is due to the interplay between the sodium influx through the channels and electrogenic effect of the Na+/K+ pumps. The NEAs sustaining is dependent on the electrogenic current of the pump allowing conjecture that the transition between NEA and SD would be dependent on the pump action. In fact, it was possible to simulate the transition to SD by decreasing pump activity. Experimentally, reducing the Na+/K+ pumps activity during the NEAs by hypoxia, the transition was observed. Conclusions: The simulation of NEAs transition to SD using a model properly designed to study the NEAs showed that the mechanisms of the model were enough to describe the SD onset. Results suggested the interplay between the influx of Na+ through the NVDC and the Na+/K+ pump electrogenic currents that sustain the NEAs and the transition to SD as well.

Disclosures: **A.G. Almeida:** None. **A.M. Rodrigues:** None. **M.F. Miranda:** None. **L.C. Santos:** None. **C.A. Scorza:** None. **F.A. Scorza:** None. **E.A. Cavalheiro:** None.

Poster

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Topic: C.07. Epilepsy

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Title: Point process modeling of human seizures

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Abstract: Epilepsy is a serious neurological disease often resulting from complex, multiscale processes. Clinical evaluation of human epilepsy routinely involves the analysis of *ictal discharges (IDs)*: transient large-amplitude changes in the electric field. IDs appear at multiple spatial scales leading up to and during seizure. These events are fundamental to diagnosis; however there is significant controversy over whether they aid or inhibit seizures. To resolve this issue and, in turn, improve epilepsy treatment will require rigorous notions of ID likelihood and causality. Descriptive measures such as spike-field coherence and spike count correlation ***may be useful for understanding simple associations, yet they are easily confounded by variables such as spike rate.*** Point process (PP) models represent a simple yet flexible way to decompose ID probability and thereby better understand seizure dynamics and anti-epileptic treatment. With this approach, the modeler recasts factors that modulate spike probability (e.g. refractory period, rhythms) as statistical effects, estimates all effect sizes from the data, and compares their mutual importance to draw physical conclusions. Here we analyze IDs recorded during 10 seizures in 4 clinical patients. We extract IDs from the microelectrode recordings via an automated algorithm and fit PP models with intrinsic ("local") and population ("long-range") effects. While similar to bivariate measures such as the correlogram and spectrum, the effect estimates capture multivariate structure and satisfy the Cramer-Rao bound. We use these PP model estimates to show: (1) IDs are highly non-random; (2) Both intrinsic and population effects are necessary to characterize ID structure; and (3) The two types of effect (local vs. long-range) act on different time scales, often in a complementary manner. For each seizure, the effect curves suggest a possible treatment plan by identifying spatially-specific patterns that could promote seizure termination.

Disclosures: G.M. Fiddyment: None. **U.T. Eden:** None. **S.S. Cash:** None. **M.A. Kramer:** None.

Poster

419. Epilepsy: Networks

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Title: Network burst dynamics under heterogeneous cholinergic modulation of neural firing properties and synaptic connectivity

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Abstract: The characteristics of synchronous network bursts, that underlie seizure-like activity, depend on intrinsic neural properties and synaptic connectivity in the network. In brain networks, both of these properties are affected by the type and levels of neuromodulators present. The expression of many of the most powerful neuromodulators, including acetylcholine (ACh), dynamically varies with behavioral state, leading to dynamic, heterogeneous changes in intrinsic neural properties and synaptic connectivity properties. In this simulation study, we investigated how heterogeneity in cholinergic modulation of neural firing properties and heterogeneity in synaptic connectivity affects the initiation and maintenance of network bursting in excitatory networks. We utilized a Hodgkin-Huxley model of cortical pyramidal cells where ACh modulation was simulated by blockade of an M-type K+ mediated current. Previous work has shown that this ACh modulation significantly alters neural firing properties such that the phase response curve (PRC) switches between Type II and Type I, thus altering the propensity for network burst synchronization. Here, we show in large-scale networks with heterogeneous ACh modulation that cells that are strongly (Type I) and weakly (Type II) modulated by ACh contribute differentially to the initiation and maintenance of network bursts. Synchronous dynamics of Type II neurons is needed for burst initiation and provides synchronizing drive to Type I cells, whereas Type I cells provide the overall activity level necessary to sustain burst firing. Characteristics of network bursts, such as burst duration and intraburst synchrony, are dependent on the cell types providing the synaptic connections in the network. The existence of network bursting depends on synaptic activity from Type II to Type I cells. Intraburst synchrony varies with the fraction of synapses between Type I cells while burst duration varies with the fraction of synapses between Type II cells. These results suggest how nonuniform neuromodulation can affect network dynamics and offer targets for intervention of synchronous activity associated with seizures.

Disclosures: **S. Knudstrup:** None. **M. Zochowski:** None. **V. Booth:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

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Title: Abnormal metabolic connectivity in the pilocarpine-induced epilepsy rat model: A multiscale network analysis based on persistent homology

Authors: H. CHOI[1], Y. KIM[2], H. KANG[1], H. LEE[1], H.-J. IM[3], D. HWANG[1], Y. LEE[4], E. E. KIM[3], J.-K. CHUNG[1], *D. LEE[5]
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Abstract: Temporal lobe epilepsy is associated with dysfunctional brain networks. Here we investigated metabolic connectivity in the pilocarpine-induced epilepsy rat model and applied a new multiscale framework to the analysis of metabolic networks of small-animal brains. [^{18}F]fluorodeoxyglucose PET was acquired in pilocarpine-induced chronic epilepsy rats and controls to yield interregional metabolic correlation by inter-subject manner. When interregional correlation of epilepsy rats and controls was compared directly, the epilepsy rats showed the reduced connectivity involving left amygdala and left entorhinal cortex. When regional graph properties were calculated to characterize abnormal nodes in the epileptic brain network, the epilepsy rats showed reduced nodal and local efficiency in left amygdala. Then, a new multiscale framework, persistent brain network homology, was used to examine metabolic connectivity with a threshold-free approach and the difference between two networks was analyzed using single linkage distances (SLDs) of all pairwise nodes. We found a tendency of longer SLDs between left insula/left amygdala and bilateral cortical/subcortical structures in the epilepsy rats.

Persistent brain network homology analysis as well as interregional correlation study implied the abnormal left limbic-paralimbic-neocortical network in the pilocarpine-induced epilepsy rat models. In conclusion, we found globally disrupted network in the epileptic brain in rats, particularly in the limbic and paralimbic structures by direct comparison, graph properties and multiscale network analysis. These results demonstrate that the multiscale and threshold-free network analysis can be used to find the network abnormality in small animal brains as a preclinical research.

Disclosures: **H. Choi:** None. **Y. Kim:** None. **H. Kang:** None. **H. Lee:** None. **H. Im:** None. **D. Hwang:** None. **Y. Lee:** None. **E.E. Kim:** None. **J. Chung:** None. **D. Lee:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: NIH Grant 7R01NS075249-04

Title: Environmental enrichment improves hippocampal networks in animals with malformations of cortical development

Authors: ***A. E. HERNAN**[1], M. LUCAS[2], K. JENKS[2], J. BARRY[1,2], M. TESTORF[2], P.-P. LENCK-SANTINI[1], G. L. HOLMES[1], R. C. SCOTT[1,3]
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Abstract: Children with malformations of cortical development (MCD) frequently present with learning and memory deficits that are extremely detrimental to the quality of life. Currently available therapeutic strategies focus on the seizures common in this patient population, however these treatments are usually ineffective at ameliorating cognitive deficits. We recently showed that environmental enrichment improves cognition in animals exposed to methylazoxymethanol acetate (MAM) at embryonic day (E) 17, an animal model that recapitulates many of the aspects of human MCD. Using this model, we sought to understand 1) the cellular and network-level alterations that occur in animals with MCD and 2) whether or not environmental enrichment improves these putative alterations in a manner consistent with improved cognition. We show for the first time that malformations of hippocampal CA1 cause changes in neuronal oscillations and

single unit activity in the hippocampus of rats with MCD. Environmental enrichment partially restores oscillatory and single unit activity in MAM animals, changes that likely underlie improvements in cognition. Taken together, these data indicate that there are network-level alterations in the hippocampus of animals with MCD and that these changes can be partially reversed by enrichment strategies. Future studies will continue to explore the cellular and network-level underpinnings of cognitive deficits in animals with MCD in order to develop effective treatment strategies for these patients.

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Poster

419. Epilepsy: Networks

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Support: NIH-NINDS R01NS079533 (WT)

NIH-NINDS K01 Career Award NS057389 (WT)

Department of Veterans Affairs, Merit Review Award (WT)

Title: Modeling of neocortical neural dynamics during human focal seizures

Authors: *E. C. HO[1,2], W. TRUCCOLO[1,2]
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Abstract: Recent advances in microelectrode array (MEA) recordings have made possible the simultaneous recordings of spiking activity in ensembles of neocortical single neurons during human focal seizures. As a result, a much more detailed picture of seizure initiation, spread and termination, has begun to emerge [1]. Here, we combine single-neuron spiking and local field potential (LFP) data obtained from 96-MEA recordings during human seizures with mathematical modeling. Our goals are to understand the specific cellular and synaptic mechanisms responsible for two types of observed seizures (spike-wave complexes--SWCs, 2-3Hz, and gamma-band, 40-60 Hz, seizures), and to determine how these mechanisms are related to the initiation and cessation of seizures. We model the neocortex under a (4mm x 4mm) 96-

MEA with excitatory and inhibitory conductance-based neurons embedded in 50-µm wide cortical minicolumns, including layers and typical connectivity. We have studied models consisting of up to 256 minicolumns (~ 1.1M neurons). Extracellular ionic dynamics include glial regulatory mechanisms and extracellular ionic diffusion processes between neighbouring neurons, while background synaptic inputs are represented by Ornstein-Uhlenbeck processes. Gamma-band like seizures resulted by combining strong mutual inhibitory synaptic couplings (gi->i), strong inhibitory to excitatory synaptic couplings (gi->e), and high background activity levels to both the pyramidal and interneuronal populations. In this case, irregular single-neuron spiking patterns coexisted with sustained narrow gamma band LFP oscillations, resembling the recorded human gamma-band seizures. Dynamics similar to SWC seizures were obtained by decreasing both mutual inhibitory coupling (gi->i) and inhibitory to excitatory coupling (gi->e). In this network model, transitions into seizure dynamics are closely associated with fluctuations in extracellular potassium concentrations. A transient increase in extracellular potassium concentration depolarizes neurons and tends to initiate seizures. Furthermore, the model also suggests that a breakdown of inhibitory synaptic conductance may be responsible for the network to transition into SWC seizures. Overall, the model captures several aspects of the rich dynamics in human focal seizures, and it is thus a promising tool for examining potential biophysical mechanisms underlying human epilepsy. 1. Truccolo W, Donoghue JA, Hochberg LR, Eskandar EN, Madsen JR, Anderson WS, Brown EN, Halgren E, Cash SS, Single-neuron dynamics in human focal epilepsy. Nat Neurosci 2011, 14:635-6432.

Disclosures: **E.C. Ho:** None. **W. Truccolo:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

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CURE (Julie's Hope Award)

Mirowski Foundation

Title: Dynamic functional reconfiguration in human epileptic networks

Authors: *A. KHAMBHATI[1], B. LITT[2], D. S. BASSETT[2]
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Abstract: For 33% of epilepsy patients seizures cannot be controlled by medication. These patients are often implanted with intracranial electrodes to localize and surgically resect seizure "foci." However, seizure freedom post-surgery is ~40% in patients without discrete lesions on brain MRI, spawning new implantable neuromodulation devices to control brain regions that generate seizures without removing them. The challenge remains to accurately define epileptic networks and their complex spatial interactions to inform which neural circuits to target with neuromodulation. We used intracranial recordings from epilepsy patients undergoing presurgical evaluation to analyze neocortical functional connectivity before and during seizures. We report a novel technique to track network reconfiguration in time and to parse these reconfiguration dynamics into distinct seizure phases. Our approach characterizes changes in gross connectivity throughout seizures and the spatiotemporal relationships between areas generating seizures and surrounding tissue. Our results indicate that epileptic networks reorganize before seizures and much less during these events. We found that seizures undergo three distinct phase transitions, each characterized by unique patterns of network connections that differ in their strength and topological extents. These findings suggest that different regions of the network are involved in initiating and terminating seizures. Collectively, our observations have important theoretical implications for understanding the spatial involvement of distributed cortical structures in seizure propagation and termination, and practical significance in determining which circuits to modulate with implantable devices.

Disclosures: A. Khambhati: None. **B. Litt:** None. **D.S. Bassett:** None.

Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

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Epilepsy Foundation Grant 222178

Title: Multiscale wave propagation during human seizures

Authors: *L.-E. MARTINET[1], O. J. AHMED[2], E. N. ESKANDAR[3], S. S. CASH[2], M. A. KRAMER[1]
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Abstract: Epilepsy is a complex multiscale disease, extending spatially from microscopic synapses to macroscopic behavioral manifestations. Seizures - the hallmark of epilepsy - exhibit multiscale spatiotemporal dynamics that remain incompletely understood. Here we study the properties of focal seizure onset, propagation and termination using a unique data set of multiscale recordings from microscopic (NeuroPort, 10x10 grid, 0.4 mm spacing) and macroscopic (8x8 grid, 10 mm spacing) subdural electrode arrays implanted in patients with pharmacoresistant epilepsy. We find voltage waves propagating along the brain surface in the local field potential (LFP), as well as in the invasive electroencephalogram (ECoG) recordings. We explore different spatiotemporal properties of these waves such as amplitude, speed, width and rate of occurrence, during the evolution of seizure from onset to termination, and how these features relate to etiology and surgical outcome. We also describe the interscale relationship between waves in the LFP and EEG recordings. We hypothesize that wave features evolve during seizure, with more consistency at seizure onset and termination, compared to the mid-seizure interval. A deeper understanding of ictal wave propagation may have clinical implications such as tailoring surgical interventions or guiding stimulation induced seizure interruption.

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Poster

419. Epilepsy: Networks

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Topic: C.07. Epilepsy

Support: NSF-1264948

Title: The effects of altering intra- and interhemispheric excitability on the bilateral propagation of epileptiform events and hemodynamic responses in rats

Authors: *H. MA, A. G. S. DANIEL, M. ZHAO, T. H. SCHWARTZ
Dept Neurolog. Surgery, Weill Cornell Med. Col., NEW YORK, NY

Abstract: Our previous studies have demonstrated that a strong relationship between the local neuronal activities and hemodynamic changes exists during ictal events in acute focal neocortical epilepsy and the hemodynamic change can be used to map the epilepsy focus. However; the neuronal-hemodynamic relationship on the contralateral hemisphere has not been described. We address this issue using simultaneous wide-field calcium and intrinsic optical imaging. Local injection of 4-aminopyridine (4-AP, 500nl, 15mM) was employed to induce acute ictal events in adult SD rats. Local injection of bicuculline methiodide (BMI, 500nl, 5mM) in either the ipsilateral or contralateral hemisphere was employed to manipulate the excitability of the neuronal network. 4-AP seizures could be recorded in a local area (propagated 2-3 mm from the injection site). The resulting hemodynamic changes could be recorded from the same area. Increased neuronal activity could also be recorded in the mirroring area of the contralateral hemisphere, with an amplitude lower than the injection side. However, the increased neuronal activity failed to induce obvious hemodynamic changes. We then injected BMI ~4mm away from the 4-AP site in the same hemisphere to promote the ictal events. A larger spatial propagation of the ictal event was observed (to the BMI injection site). On the contralateral hemisphere, more robust neuronal activity was also observed which induce a less pronounced hemodynamic change than the seizure side. In some cases, hemodynamic changes were barely detectable. BMI was then injected in the contralateral hemisphere to increase the neuronal activity. The ictal event could be recorded from both hemispheres with similar duration and spatial propagation. The hemodynamic changes could be reliably recorded from the BMI side but the amplitude was lower than the 4-AP side. Our data suggest that the inhibition on the mirroring side limits the bilateral propagation of local seizures. Neurovascular coupling may not be the only factor that manipulates the hemodynamic changes when a hyper-excited ictal focus exists on the contralateral hemisphere. The hemodynamic difference between the two hemispheres could serve as a biomarker to distinguish the primary seizure focus from the mirror focus.

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Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Topic: C.08. Ischemia

Support: NIH grants R41NS073378

AHA Established Investigator Award

AHA Postdoctoral Fellowship (12POST12080252)

Title: Axonal injury and behavioral deficits after ischemic stroke in the sensorimotor cortex of channelrhodopsin-2 transgenic mice

Authors: M. SONG[1], X. GU[1], L. WEI[1], *S. YU[2]
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Abstract: Expression of the light-activated channelrhodopsin-2 (ChR2) protein is an excellent tool to conduct optogenetic stimulation and functional mapping of the cortical circuits in the brain. In an ischemic stroke investigation, we used the line 18 transgenic mice expressing ChR2 that was fused with Yellow Fluorescent Protein (ChR2-YFP) under the control of the mouse thymus cell antigen 1 (Thy1) promoter. ChR2-YFP expresses at high levels in motor and sensory neurons, as well as subsets of central neurons but not in non-neural cells. Axons are brightly fluorescent all the way to the terminals when imagined under a fluorescent microscope. In this study, we characterized the behavioral deficits and axonal injury three or more days after a focal ischemia induced by ligations of distal branches of the right middle cerebral artery. A home cage monitoring system was applied to analyze behavioral and functional changes of stroke animals with little human intervention. It was shown that stroke caused dramatic reductions in travel distance, body stretching, sniffing and chewing activities. The time hanging vertically and hanging cuddled significantly decreased, indicating deficits in the sensorimotor function associated with forepaw activities. The adhesive removal behavioral test further indicated the specific sensorimotor deficit of the left paw that is affected by the injured sensorimotor cortex. Imaging of the brain sections showed that the density of YFP-labeled axons was decreased in the ischemic region compared with normal animals. Immuno-fluorescent staining of amyloid precursor protein (APP) shows accumulated APP in the injured axonal terminals. Axonal swelling and demyelination was confirmed by electron microscopy. Optogenetic stimulation is being applied to promote functional recovery in this stroke model.

Disclosures: **M. Song:** None. **S. Yu:** None. **X. Gu:** None. **L. Wei:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

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Program#/Poster#: 420.02/W20

Topic: C.08. Ischemia

Support: CIHR/HSFC Grant CIF99472

Title: Adulthood protein-energy malnutrition augments stroke-induced abnormalities in forelimb function in rats

Authors: *M. ALAVERDASHVILI, P. G. PATERSON
Neurosci. Res. Group and Col. of Pharm. and Nutr., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Pre-existing protein-energy malnutrition (PEM) affects 12-19% of elderly stroke patients. Worse outcomes (e.g. mortality) in stroke patients due to PEM suggest that PEM is an important co-morbidity factor. Nevertheless, the impact of PEM is not clear for non-fatal post-stroke outcome such as hand use in daily activities. Thus, the aim of this study was to explore the effect of pre-existing adulthood PEM on stroke-induced abnormalities in forelimb use in rats. Male, Sprague-Dawley rats (12 week-old) fed either with a purified diet containing 0.5% protein (PEM: n=12) or the control diet (CONT: n=10) for 4 weeks were exposed to focal ischemia (I) to the motor cortex or sham surgery (SHAM). Photochemically-induced thrombosis targeted the caudal region of the forelimb area of motor cortex. PEM-I, CONT-I, PEM-SHAM and CONT-SHAM rats were tested for skilled “hand” function in a horizontal ladder walking task and for gross “hand” function in a cylinder task acutely after stroke. Rats exposed to focal ischemia made significantly more errors with the affected (contralateral to brain injury) forelimb during crossing the ladder ($p<0.05$). Most importantly, PEM augmented ischemia-induced abnormalities in forelimb placement accuracy ($p<0.05$). PEM-I rats fell between rungs after the affected limb slipped off the rung, whereas CONT-I rats adopted a compensatory strategy and replaced the limb from one rung to another rung when the affected forelimb was placed inaccurately. PEM also amplified the stroke-induced deficit in the cylinder task. PEM-I rats showed more bias than CONT-I rats for contacting the ground with the unaffected forelimb (ipsilateral to brain injury) after episodes of rearing ($p<0.05$). The findings of this study suggest a definitive link between pre-existing PEM and motor function in the acute period following ischemic stroke. (Funded by CIHR and HSFC)

Disclosures: M. Alaverdashvili: None. **P.G. Paterson:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Topic: C.08. Ischemia

Support: NIH Grant HL63290

Title: An activated protein C analog promotes survival, migration and differentiation of human neural progenitor cells in ischemic mouse cerebral cortex

Authors: *Y. WANG[1], Z. ZHAO[1], G. SI[1], S. REGE[1], J. GRIFFIN[2], B. ZLOKOVIC[1]
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Abstract: Despite intensive research efforts, few effective treatments are available for stroke. Cell therapy offers the possibility of improving neurological deficits after stroke. However, animal studies have shown that low survival rate of transplanted stem cells in the brain precludes long-term beneficial therapeutic effects. 3K3A-APC, a recombinant analog of activated protein C (APC), exerts neuroprotective effects after an acute or chronic injury in the brain. Our recent *in vitro* studies showed that 3K3A-APC stimulated human neural progenitor cells (NPCs) proliferation, migration and differentiation and production of neuronal cells. It is unknown whether human 3K3A-APC can influence survival, proliferation and differentiation of transplanted NPCs in ischemic brain in mice. Ischemic stroke was induced by distal middle cerebral artery occlusion (dMCAo). In this stroke model striatum remains intact and damage is limited to cortex. NPCs were transplanted into ischemic cortex 7 days after dMCAo. Human 3K3A-APC (0.2 mg/kg, i.v.) was administered at 7, 9, 11, and 13 days after stroke. We found that 3K3A-APC significantly enhanced the proliferation of NPCs and potentiated their differentiation into neuronal lineage after transplantation. We also show that the combined treatment with 3K3A-APC and NPCs significantly improved motor function as early as 2 weeks after transplantation. These findings suggest that 3K3A-APC may boost beneficial effects of NPCs transplantation therapy for ischemic stroke.

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Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Title: Evaluating the clinical significance of serum Progranulin levels in patients with acute cerebral infarction

Authors: *J. LI[1], S. LIU[1], Y. ZHAO[1], Z. Z. WEI[1], Y. ZHANG[1], Y. LV[1], S. YU[2], L. WEI[2]
[1]Dept. of Neurology, Beijing Friendship Hospital, Capital Med. Univ., Beijing Friendship Hosp., Beijing, China; [2]Anesthesiology/Neurology, Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Objective: Inflammatory factors are considered to be major contributors to systematic inflammatory diseases, metabolic syndromes and cerebral infarction (CI).Progranulin (PGRN) is an autocrine growth factor which plays a critical role in a variety of patho-physiological processes, including inflammation, cutaneous wound healing and tumorigenesis. PGRN blocks the downstream signaling of TNF through directly binding to its receptors. We attempted to study the serum PGRN level in patients with CI and its role in the disease development. **Subjects and Methods**: Patients diagnosed with CI went through brain CT or MRI for further verification. A total of 35 patients and 25 age-and-gender matched control subjects were enrolled and subgrouped by onset time, infarction size, and the degree of artery stenosis were measured for each patient. Demographic data, medical history, clinical features, risk factors and imaging of all subjects were collected and analyzed. ELISA was used to test serum PGRN levels. Correlation analyses was performed to investigate the association between PGRN levels and the patients' disease features. **Results**: The serum PGRN levels increased significantly in CI patients (41.44±10.62ng/ml) compared with control subjects (35.25±7.83ng/ml) ($P<0.05$). However, there were no significant differences between the subgroups of disease onset, infarct size, risk factors and degrees of artery stenosis. Moreover, PGRN levels were not correlated with neurological impairment levels. **Conclusion**: The serum PGRN levels were increased in patients with acute cerebral infarction. However no differences were observed in subgroup analysis of disease onset, infarct size, risk factors and degrees of artery stenosis. We suggested that PGRN might be involved in the inflammatory processes and further studies would be required to investigate the mechanism involved in cerebral infarction.

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Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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NIH T32 NS007449

American Heart Association Predoctoral Fellowship

NINDS Ruth L. Kirschstein NRSA F31NS086431-01

Title: Activity-dependent formation of new premotor connections in the post-stroke brain

Authors: *E. H. NIE[1,2], G. COPPOLA[3], S. T. CARMICHAEL[2]
[2]Neurol., [3]Psychiatry & Biobehavioral Sci; Neurol., [1]UCLA, Los Angeles, CA

Abstract: Each year, five million stroke survivors worldwide are left with permanent disabilities for which there are no medical treatments. However, human clinical trials have shown that constraint-induced movement therapy (CIMT), a behavioral paradigm for focused overuse of the injured limb, results in significant and lasting motor improvements after stroke. The goal of the current project is to understand how limb overuse shapes cortical reorganization after ischemic stroke, and how specific gene systems drive this critical repair process. Our work has previously identified a molecular growth program that is triggered after stroke to promote axonal sprouting in the surviving brain. To study how limb overuse shapes sprouting, we utilize a mouse model of stroke and forelimb overuse that approximates human CIMT. In this model, fluorescent neuroanatomical tracers were used to quantitatively map cortical neurons that connect to premotor cortex upon limb overuse. We find that recovery involves the activity-dependent formation of axonal connections between premotor cortex and retrosplenial cortex (RSC), a higher-order parietal area involved in spatial integration and memory. The finding was significant and replicated across two independent experimental cohorts ($p<0.05$, Hotelling's T test). Furthermore, C5 and T10 retrograde corticospinal tract tracing confirm that the activity-induced connections are indeed distinct from known layer V corticospinal motor regions. In a

second tier of studies, the tracer-labeled cells in RSC and matched control neurons were FACS-isolated, and high quality RNA (RIN=7) was extracted for RNA-Seq. Preliminary pathway analyses indicate that limb overuse induces canonical signaling in calcium signaling, cell cycle, and Ephrin A pathways. A top network hit was also found in cell growth and proliferation, tissue development, and cell morphology. The RSC-premotor circuit is characterized by circuit-specific expression of activity-induced and growth-related genes. Moreover, a relatively small group of 162 genes was identified as differentially regulated by limb overuse. The top candidate genes have been statistically prioritized (FDR<0.1, p<0.005) for in-vitro neuronal outgrowth assays using P3 primary cortical neurons. Select candidates that increase neuronal outgrowth will advance to in-vivo lentiviral and siRNA mediated functional studies within the RSC-premotor circuit mapped in the first phase of this study. These ongoing studies will shed light on how injury and activity-dependent molecular pathways may converge in CIMT, and potentially provide targets to enhance endogenous mechanisms of brain repair after stroke.

Disclosures: E.H. Nie: None. **G. Coppola:** None. **S.T. Carmichael:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Program#/Poster#: 420.06/W24

Topic: C.08. Ischemia

Title: Effects of Memantine on NO production, hydroxyl radical metabolism during cerebral ischemia and reperfusion in mice

Authors: *T. SASAKI, T. FURUYA, Y. ITO, M. YAMASATO, R. NISHIOKA, A. TANAKA, N. ARAKI
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Abstract: Methods: C57BL/6 mice [n=15] were used. Memantine 25 μmol/ kg was given in 5 mice 30 minutes before ischemia, and others were contol group. Both NO production and hydroxyl radical metabolism were continuously monitored by *in vivo* microdialysis. Microdialysis probes were inserted into the bilateral striatum. The *in vivo* salicylate trapping method was applied for monitoring hydroxyl radical formation via 2,3 dihydroxybenzoic acid (DHBA), and 2,5-DHBA. A Laser doppler probe was placed on the skull surface. Forebrain cerebral ischemia was produced by occlusion of both common carotid arteries for 10 minutes. Levels of NO metabolites, nitrite (NO2-) and nitrate (NO3-), in the dialysate were determined

using the Griess reaction. The temporal expression of NOS in transient forebrain cerebral ischemia was investigated by western blot analysis and immunohistochemistry. Results: (1) Blood pressure: There were no significant differences between the groups. (2) Cerebral blood flow (CBF): There were no significant differences between the groups. (3) NO2- ; Memantine group (120.9±5.00%; mean±SD) showed significantly higher than that of the control group (88.5±18.0) after repurfusion 60 minutes ($p<0.05$). (4) NO3-; Memantine group (97.2±10.1%; mean±SD) showed significantly higher than that of the control group (65.3±21.0) at ischemia ($p<0.05$). (5) 2,3-DHBA; Memantine group (90.7±2.90%; mean±SD) showed significantly lower than that of the control group (99.5±2.66) at ischemia, after reperfusion 20, 80-120 minutes ($p<0.05$). Conclusion: These *in vivo* data suggest that Memantine effects on NO metabolites and hydroxyl radical production in mice, and may have neuroprotective effect against cerebral ischemic injury.

Disclosures: T. Sasaki: None. **T. Furuya:** None. **Y. Ito:** None. **M. Yamasato:** None. **R. Nishioka:** None. **N. Araki:** None. **A. Tanaka:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.07/W25

Topic: B.04. Ion Channels

Support: PJ009835032014

NRF-2008-0061888

NRF-2012R1A1A2009219

Title: Tyrosine phosphorylation of Kv2.1 channel contributes to neuronal cell death during brain ischemia

Authors: *M. SONG, K.-S. PARK
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Abstract: Oxidative stress induces neuronal apoptosis and is involved in brain ischemia. Oxidant induced apoptosis enabling intracellular K+ efflux is mediated by improvement of Kv2.1 channel activity. It has been shown that increased channel activity is triggered by tyrosine phosphorylation of Kv2.1 and through the Src kinase. However, the functional role of Kv2.1

tyrosine phosphorylation in brain ischemia is not fully known. Here we provide the evidences that the oxidative stress-induced brain ischemia is regulated by tyrosine phosphorylation of Kv2.1. We found that the tyrosine phosphorylation levels of Kv2.1 were increased by oxidant induced neuronal ischemia. In a brain ischemia model, the tyrosine phosphorylation of Kv2.1 is also increased after brain ischemia. A sustained increase of tyrosine phosphorylation was observed for at least 2h and peaked at 45min of reperfusion. Our results show that the tyrosine phosphorylation of Kv2.1 channel play a critical role in regulating neuronal ischemia, and may be a potential therapeutic target for brain ischemia.

Disclosures: **M. Song:** None. **K. Park:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.08/W26

Topic: C.08. Ischemia

Title: Stem cell neural repair after white matter stroke

Authors: *I. L. LLORENTE[1], J. CINKORNPUMIN[2], W. E. LOWRY[2], S. T. CARMICHAEL[3]
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Abstract: Subcortical white matter stroke (WMS) constitutes up to 25% of all stroke subtypes and is the second leading cause of dementia. In this study we developed a new WMS model which mimics many aspects of the human disease. This model has been adapted to the immunodeficient NSG mouse to avoid any risk of immune rejection. There is no therapy for white matter stroke. However, cell transplantation is emerging as a viable therapy to restore neurological function after white matter stroke. Human induced pluripotent stem cells (iPSCs) have been used as an appealing cell source for cell transplantation to repair neuronal networks disrupted by ischemic stroke. However, neural precursor cells might not be the preferred cell therapy for white matter damage. We tested 2 cell types that may produce white matter repair. iPS-neural precursor cells (NPCs) can differentiate into neurons, astrocytes and to a lesser extent oligodendrocytes. iPS-glial restricted progenitor cells (GRPs) will differentiate mostly into astrocytes but may more be bipotential for astrocyte and oligodendrocytes . To characterize how these cell types response to white matter stroke overtime (1, 7, 15 days and 2 months) and how they differentiate in this white matter stroke environment we determined the inflammatory

response (iba1), astrocyte activation (GFAP), axonal/myelin loss and repair (NF200/MBP) as well as the their differentiation potential (oligodendrocytes, astrocytes and neurons) and the endogenous migration of immature neurons (DCX). Also, we have developed mouse MRI imaging protocols that visualize the white matter stroke, with the same imaging sequences that visualize white matter stroke in humans, making possible *in vivo* tracking of white matter repair with cell transplant, and holding the promise of developing a biomarker for stem cell-mediated neural repair in this disease.

Disclosures: I.L. Llorente: None. **S.T. Carmichael:** None. **W.E. Lowry:** None. **J. Cinkornpumin:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.09/W27

Topic: C.08. Ischemia

Support: HKU UDF

Title: Vulnerable vasculature and increased inflammation contribute to exacerbation of transient focal ischemia in a genetic mouse model of type 1 diabetes

Authors: *A. C. LO[1,2], A. K. W. LAI[1]
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Abstract: PURPOSE: Epidemiological studies showed that type 1 diabetic patients are more prone to cerebrovascular mortality from stroke and the median survival is only half when compared with those in the general population. It has been suggested that type 1 diabetes is a risk factor for stroke; however, the underlying mechanisms are still unclear. In the current study, we aim to elucidate the potential mechanisms contributing to the exacerbation. METHOD: $Ins2^{Akita/+}$ mice, a type 1 diabetic murine model, and their wildtype ($Ins2^{+/+}$) littermates at 12 weeks of age were challenged with experimental stroke induced by middle cerebral artery occlusion (2h ischemia and 2h or 22h reperfusion). Survival was recorded at selected intervals and neurological deficits were accessed at the end of reperfusion. Brain slices were stained with 2, 3, 5-triphenyltetrazolium chloride for estimating the infarct area, infarct volume, hemispheric swelling, and hemorrhagic area. Western blot analyses were performed to compare blood vessel

integrity (ZO-1, VEGF, MMP-2 and MMP-9) and inflammatory response (pErk and p-p38) in the infarct core and penumbra region. RESULTS: After 2h of reperfusion, the neurological deficits, infarct area and infarct volume were significantly increased in the $Ins2^{Akita/+}$ mice. A higher mortality rate and a shorter survival period were also observed. Hemorrhage was significantly increased and further advanced with longer reperfusion. At 2h after reperfusion, VEGF and pErk were remarkably up-regulated and ZO-1 was down-regulated in the $Ins2^{Akita/+}$ mice. A trend of increase in MMP-2, MMP-9 and p-p38 were also observed. At 22h after reperfusion, the increased expressions of p-Erk and p-p38 persisted at a significant level. CONCLUSION: We showed that induction of transient focal ischemia in $Ins2^{Akita/+}$ mice could mimic the clinical observations of high mortality and shortened survival in type 1 diabetic patients upon stroke. The increased hemorrhage together with VEGF up-regulation and ZO-1 decrease indicated that blood vessel integrity was more vulnerable in the $Ins2^{Akita/+}$ mice. The effect of hemorrhagic transformation was observed as early as 2 h after reperfusion and was further provoked with longer reperfusion. Inflammatory response may also play an important role in the exacerbation of ischemic injury in $Ins2^{Akita/+}$ mice.

Disclosures: A.C. Lo: None. **A.K.W. Lai:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

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Program#/Poster#: 420.10/W28

Topic: C.08. Ischemia

Support: QREN

FEDER

FCT [PTDC/NEU-NMC/0198/2012 and PEst-C/SAU/LA0001/2013-2014

Title: Alterations of GABAA receptor trafficking in the Oxygen-Glucose Deprivation *in vitro* model of cerebral ischemia

Authors: M. MELE[1], M. ASPROMONTE[1,2], *C. B. DUARTE[1]
[1]Ctr. Neurosci Cell Biol, Univ. Coimbra, Coimbra 3004-517, Portugal; [2]Biol. and Envrn. Studies, Univ. of Sannio, Benevento, Italy

Abstract: Cerebral ischemia is a pathological condition caused by insufficient blood supply to the brain, which causes an imbalance between excitatory/inhibitory neurotransmission and excitotoxic neuronal death. The activity of neuronal networks in the CNS is mainly determined by the balance between glutamatergic and GABAergic neurotransmission, which are up- and down-regulated, respectively, during ischemic insults. In contrast with the role of glutamate in ischemic damage, which is largely documented, the alterations in inhibitory neurotransmission remain poorly understood. *In vivo* and *in vitro* studies have shown a downregulation of GABAergic neurotransmission in the ischemic brain, both at the pre- and post-synaptic levels. GABAA receptors (GABAAR) are the major players in fast synaptic inhibition in the CNS, and a downregulation of the surface expression of GABAARs has been shown in *in vivo* and *in vitro* models of ischemia. Previous results from our lab showed that Oxygen-Glucose Deprivation (OGD) induces the internalization of GABAAR via clathrin dependent endocytosis. Furthermore, our data showed that the OGD-induced receptor dephosphorylation and consequent internalization contributes to neuronal cell death, as demonstrated using a phospho-mutant of the β3 GABAAR subunit. These evidence indicate that the number of GABAAR at the cell surface and receptor internalization play a key modulatory role in the induction of ischemic cell death, but the receptor fate after internalization in ischemic conditions have not been elucidated. In the present work we investigated the molecular mechanisms determining GABAAR sorting upon internalization in cultured hippocampal neurons subjected to the OGD *in vitro* model of ischemia. Under physiological conditions, following internalization, GABAARs are rapidly recycled back to the plasma membrane or targeted for lysosomal degradation. The decision regarding the sorting of endocytosed GABAARs depends on the interaction of GABAAR β1-3 subunits with huntingtin-associated protein 1 (HAP1). We found that OGD (70 min) reduces the recycling rate of GABAAR back to the plasma membrane when assessed after 20 min of internalization by the antibody-feeding assay. The same period of OGD also decrease the interaction of GABAAR with the HAP1 proteins evaluated by co-immunoprecipitation assay. Longer periods of OGD also induced a calpain-mediated cleavage of HAP1. Overall, we propose a new model in which the increase in GABAAR internalization and the impairment of the recycling mechanisms are key steps for GABAergic down-modulation during cerebral ischemia, contributing to excitotoxicity and consequent neuronal cell death.

Disclosures: **M. Mele:** None. **C.B. Duarte:** None. **M. Aspromonte:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.11/W29

Topic: C.08. Ischemia

Title: Endothelial progenitor cells protect ischemic cell damage after cerebral infarction

Authors: *T. NAKAYAMA[1,2], E. NAGATA[2], H. MASUDA[3], S. KOHARA[2], N. YUZAWA[2], Y. TAKAHARI[4], T. ASAHARA[3], S. TAKIZAWA[2]
[1]Tokai Univ., Isehara, Japan; [2]Dept. of Neurol., [3]Dept. of Regenerative Med. Sci., [4]Educ. and research supporting center, Tokai Univ. Sch. of Med., Isehara, Japan

Abstract: Background: Endothelial Progenitor Cell (EPC) was reported to enhance repairing and regenerating neurovascular units. So far many papers have reported repairing and regenerating experiments using EPCs derived from bone marrow, spleen, or peripheral blood. However, the results were not always satisfied. Recently, we succeeded to get higher grade quality EPCs using a novel colony assay system which we have developed (Masuda H, et al, 2011). In the present study, we used this novel EPC colony assay system, and evaluated EPC effects on ischemic stroke model in mice. Materials and methods: We made 27 ischemic stroke model mice (10 weeks male nude mice) with permanent middle cerebral artery occlusion (MCAO). We injected PBS as control and EPC derived from peripheral blood, or cultured EPC into external carotid artery 24 hours after MCAO. At 2 weeks after MCAO, we took the brains and investigated time-lapse physiological parameters including cerebral blood flow and immunohistochemistry against some anti-vasculogenetic factor antibodies. Results: Either EPC derived from peripheral blood or cultured EPC didn't decrease ischemic lesions. On the other hand, cultured EPC increased the cerebral blood flow ratio on ischemic hemisphere. In the mice which were injected with cultured EPCs, the number of CD31+ and eNOS positive cells were increased compared to controls. Conclusions: Those results indicate cultured human EPCs possibly leading angiogenesis on nude mice through the humoral immunity. Those cultured EPCs could enhance repairing and regenerating neurovascular units after ischemic stroke.

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Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.12/W30

Topic: C.08. Ischemia

Support: BK21 program

Title: Critical role of E2-25K/HIP2 in ischemic injury

Authors: E. JEONG[1], S. SONG[2], *Y.-K. JUNG[1]
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Abstract: Cerebral ischemia is a major cause of mortality and disability but there is no clear damage mechanism as well as no treatment or prevention. In this study, we report that E2-25K/HIP2, an E2-conjugating enzyme involved in ubiquitin proteasome system, modulates oxygen-glucose deprivation (OGD)/reoxygenation-induced cell death in neuronal cells. We found that the expression of E2-25K/HIP2 is induced in neuronal cells during OGD/reoxygenation. Knockdown of E2-25K/HIP2 expression protects against OGD/reoxygenation-induced neuronal cell death, while ectopic expression of E2-25K/HIP2 stimulates the cell death. This stimulation effect of E2-25K/HIP2 is dependent on the enzyme activity. We also observed the persistent expression of HIF-1alpha during the reoxygenation after OGD in E2-25K/HIP2-deficient neuronal cells. Compared to control mice, cerebral infarction lesion in E2-25K/HIP2 KO mice was ameliorated in a mouse ischemic model of transient middle cerebral artery occlusion (tMCAO)/reperfusion. Further, treatment of wild-type mice with N0001 compound which inhibits the enzyme activity of E2-25K/HIP2 *in vitro* compromises the cerebral infarction volume under tMCAO/reperfusion. In conclusion, E2-25K/HIP2 is critical in OGD/reoxygenation-mediated cell death.

Disclosures: **E. Jeong:** A. Employment/Salary (full or part-time):; BK21 program. **Y. Jung:** None. **S. Song:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

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Program#/Poster#: 420.13/W31

Topic: C.08. Ischemia

Support: NIH-NINDS-R21-NS081261

ATRM Division of Johnson & Johnson

Title: Effect of a cell therapy on neuropathological and neurorestorative processes following ischemic damage in rhesus monkey motor cortex

Authors: *M. E. ORCZYKOWSKI[1], M. L. MCBURNIE[2], F. MORTAZAVI[1], D. L. ROSENE[1], T. L. MOORE[1]
[1]Dept. of Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [2]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Many experimental agents have shown potential as treatments for stroke in rodent models. However, the only therapy to have gained FDA approval to treat stroke in humans is tissue plasminogen activator (tPA) that dissolves clots and restores blood flow with efficacy only if administered within three hours of symptom onset. Following stroke, with or without tPA treatment, patients are often left with permanent residual impairment. CNTO 0007 is an investigational cell product containing human umbilical tissue-derived cells that was tested in our established non-human primate model of cortical ischemia for efficacy in enhancing recovery and reducing the permanent impairment of stroke. To test this, rhesus monkeys were trained on a quantifiable battery of fine motor tasks. After reaching asymptotic performance, the primary motor cortex was mapped using electrophysiological stimulation to identify the hand representation. Then the cortical blood vessels of only the hand representation were disrupted creating a reproducible ischemic lesion that impaired only the contralateral hand. The next day, CNTO 0007 (10M cells/kg) or vehicle control was administered intravenously. Subsequently monkeys were re-tested on the fine motor battery. Results demonstrated that treated monkeys had a significant degree of recovery of fine motor function and strength of the hand in the two weeks following surgery. During long-term (14 weeks) behavioral testing, there was significant improvement in the time to retrieve a food reward and the quality of grasp pattern in treated monkeys. To assess possible mechanisms by which CNTO 0007 facilitates post-stroke recovery, both neuropathological and neurorestorative processes are being investigated in post-mortem tissue. Using immunohistochemistry, we are quantifying activated microglia (LN-3), astrocytosis (GFAP), oxidative stress (4-HNE), and sprouting (GAP43) in premotor and primary motor cortices ipsilateral to the ischemic damage. Qualitative analysis of LN-3 revealed a decrease in activated microglia in tissue from monkeys treated with CNTO 0007. The quantitative results of the immunohistochemical assays of LN-3, GFAP, 4-HNE, and GAP43 will help us to better understand the role of CNTO 0007 in the neuropathological and neurorestorative processes following stroke.

Disclosures: M.E. Orczykowski: None. **M.L. McBurnie:** None. **F. Mortazavi:** None. **D.L. Rosene:** None. **T.L. Moore:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.08. Ischemia

Support: Canadian Institutes of Health

Heart and Stroke Foundation of Canada

Michael Smith Foundation for Health Research

Canadian Foundation for Innovation

Title: Longitudinal *in vivo* imaging of thalamocortical projections after stroke

Authors: *K. A. TENNANT, S. L. TAYLOR, C. E. BROWN
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Abstract: The large majority of stroke survivors must cope with chronic disability often affecting the upper limbs. In order to regain sensory functions after stroke, surviving neural circuits must re-organize and make new connections. Axonal projections from the thalamus are the primary source of afferent sensory inputs to the cortex, and likely play a role in recovery of function. Currently it is unknown how these projections change over time *in vivo*, after stroke. We hypothesized that thalamocortical innervation of the peri-infarct cortex is disrupted by stroke, but undergoes extensive structural remodelling that supports recovery of function. Adult C57BL/6 mice received pressure injections of adeno-associated virus (AAV-GFP) into the ventroposteriolateral nucleus of the thalamus, which sends projections to primary somatosensory cortex (S1). Immediately following virus injection, an imaging window was implanted over forelimb and hindlimb S1 (FLS1 and HLS1). Axon terminals and cerebral vasculature were imaged *in vivo* using two-photon microscopy before and at various times after photothrombotic stroke in FLS1 to assess acute and long-term changes in structure. Preliminary data indicate that, surprisingly, a subset of thalamocortical axons survived within the infarct core, but underwent branch retraction and terminaux bouton loss. In peri-infarct cortex, terminaux boutons underwent a high rate of turnover in the first week after stroke, followed by a period of bouton stabilization. Overall, peri-infarct thalamocortical axons seem relatively resilient to the effects of small focal ischemia, maintaining branches and boutons, which may provide a scaffold for functional recovery after stroke. Current studies are underway to determine the dynamics of en passent boutons after stroke.

Disclosures: K.A. Tennant: None. **S.L. Taylor:** None. **C.E. Brown:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Topic: C.08. Ischemia

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Edith and Lew Wasserman Fund for Undergraduate Support

Title: Changes in Axin2 expression following white matter ischemic stroke in mice

Authors: *M. E. REITMAN, S. ROSENZWEIG, T. CARMICHAEL
UCLA, Los Angeles, CA

Abstract: Stroke is a debilitating neurological disorder which affects approximately fifteen million people yearly world-wide and is the leading cause of adult long-term disability. Cortical ischemic stroke and the mechanisms by which neuronal death and repair occur has been a key area of neuroscientific research. However, subcortical ischemic stroke in white matter, which accounts for approximately 25% of all stroke subtypes, has not been as thoroughly studied, and the mechanisms underlying damage, repair and recovery in white matter stroke (WMS) are still poorly understood. Recently, our lab utilized a unique mouse model of WMS and RNAseq transcriptome analysis to identify differential expression of specific proteins in peri-infarct white matter following WMS. The current study characterized the temporal and spatial expression, as well as the cellular origin of one of the identified proteins -- Axin2, which acts as part of the β-catenin destruction complex to inhibit Wnt signaling. WMS was produced in mice via microinjections of the endothelial nitric oxide synthetase inhibitor N5-(1-iminoethyl)-L-ornithine (L-NIO) at three points along the white matter ventral to the forelimb motor cortex. Immunostaining for Axin2 and markers of white matter cell types was performed at critical time-points following WMS. Axin2 was found to be up-regulated outside the infarct core at both 7 day and 2 month time points post-stroke in a population of subcortical fibrous astrocytes. This suggests a late response to WMS which may facilitate reparative responses to WMS, possibly through disinhibiting the maturation of oligodendrocyte progenitor cells. To further examine the regulation and mechanism of action of Axin2, current experiments aim to examine the changes in Axin2 expression in white matter oligodendrocytes and to explore the impact of Axin2 expression on oligodendrocyte maturation and remyelination following WMS. These will further

our understanding of the cellular and molecular response to WMS and may help identify targets for therapeutic intervention following WMS in humans.

Disclosures: **M.E. Reitman:** None. **S. Rosenzweig:** None. **T. Carmichael:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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NINDS Grant 1 P50 NS44283-01

American Heart Association Fellow to Faculty Award

Title: Gene expression in peripheral immune cells following cardioembolic stroke is sexually dimorphic

Authors: *B. STAMOVA[1], G. JICKLING[1], B. ANDER[1], X. ZHAN[1], D. LIU[1], R. TURNER[1], C. HO[1], J. KHOURY[2], C. BUSHNELL[3], A. PANCIOLI[4], E. JAUCH[5], J. BRODERICK[6], F. R. SHARP[1]
[1]MIND Institute-Wet Lab., UC Davis Med. Ctr. MIND Inst., Sacramento, CA; [2]Cincinnati Children's Hosp. Med. Center, Univ. of Cincinnati, Cincinnati, OH; [3]Neurol., Wake Forest Univ. Med. Ctr., Winston-Salem, NC; [4]dDepartment of Emergency Medicine, Univ. of Cincinnati, Cincinnati, OH; [5]Div. of Emergency Medicine, Med. Univ. of South Carolina, Charleston, SC; [6]fUniversity of Cincinnati Neurosci. Institute, Dept. of Neurol., Cincinnati, OH

Abstract: Epidemiological studies suggest that sex has a role in pathogenesis of cardioembolic stroke. Since stroke is a vascular disease, identifying sexually dimorphic gene expression changes in blood leukocytes can inform on sex-specific risk factors, response and outcome biology. We aimed to examine the sexually dimorphic immune response following cardioembolic stroke by studying differential gene expression in peripheral white blood cells.

Blood samples from patients with cardioembolic stroke were obtained at ≤3 hours (prior to treatment), 5 hours and 24 hours (after treatment) after stroke onset (n=23, 11 Female, 12 Male; 69 samples) and compared with vascular risk factor controls without symptomatic vascular diseases (n=23, 11 Female, 12 Male; 23 samples). mRNA levels were measured on whole-genome Affymetrix microarrays. Differentially expressed genes were identified by Mixed Effects ANCOVA (FDR $p \leq 0.05$, $|\text{fold change}| \geq 1.2$). There were more up-regulated than down-regulated genes in both sexes, and females had more differentially expressed genes than males following cardioembolic stroke. Sexually dimorphic gene expression was noted for genes in cell death and coagulation pathways and for genes specifically expressed by neutrophils, monocytes and megakaryocytes. Distinct temporal immune profile following cardioembolic stroke was observed with immune response pathways over-represented at ≤3, 5 and 24h after stroke in female subjects but only at 24h in males. This is particularly relevant since the immune system can contribute to risk of stroke and to damage and/or to repair processes following stroke. These sex differences could also contribute to some of the reported sexual dimorphic features of stroke, such as age at stroke and response to treatment. Neutrophil-specific genes were differentially expressed at 3, 5 and 24h in females but only at 5h and 24h in males. In stroke neutrophils have recently been shown to have important roles in clotting and atherosclerosis, their increased numbers correlated with risk of having an ischemic stroke and myocardial infarction as well as with poor outcomes and increased mortality. The greater percentage of neutrophil-specific transcripts in females compared to males at early times following stroke in this study could relate to reported increased rate and morbidity of cardioembolic stroke in females. In summary, this study provides additional evidence for sexually dimorphic nature of cardioembolic stroke and can guide search for sex-specific treatments and prevention. Future studies are needed to confirm findings, to determine how they relate to risk and outcome, and to compare to other causes of ischemic stroke.

Disclosures: **B. Stamova:** None. **G. Jickling:** None. **B. Ander:** None. **X. Zhan:** None. **D. Liu:** None. **R. Turner:** None. **C. Ho:** None. **J. Khoury:** None. **C. Bushnell:** None. **A. Pancioli:** None. **E. Jauch:** None. **J. Broderick:** None. **F.R. Sharp:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Topic: C.08. Ischemia

Support: National Health and Medical Research Council-Research Grant

Stroke Foundation-Project grant

Title: Ephrins are responsible for differing astrocytic response in the post-ischemic infant and adult primate brain

Authors: *L. TEO, J. BOURNE
AUSTRALIAN REGENERATIVE MEDICINE INSTITUTE, CLAYTON, Australia

Abstract: Reactive astrogliosis initiated after CNS injuries results in the formation of a glial scar, a potent inhibitor of repair. The severity of glial scarring is more profound following adulthood CNS injuries vs. comparable early-life injury in primates, correlating to the greater potential for functional sparing and regeneration following early-life vs. adulthood CNS injuries. The mammalian receptor tyrosine kinase EphA4 is a guidance cue that plays important roles throughout the developing and injured CNS. Eph-A4 signaling is crucial for activation and regulation of reactive astrocyte (RA) activity after injury and in glial scar formation. However, less is known about the ephrin ligands required to trigger EphA4 signaling and their specific downstream effects following CNS injuries. We hypothesize Eph/ephrin expression after homologous focal neocortical ischemia differs between infant and adult primate brains, contributing to diverging RA responses and severity of glial scarring. Focal ischemia was induced in infant (P14) and adult (n=4) marmosets (Callithrix jacchus) through endothelin-1 (ET-1) induced calcarine artery occlusion. Brains were harvested at 1 and 21 days post ischemia (DPI). Western blot screens revealed differences in ephrins responses after injury. In infants, ephrinA1 was upregulated acutely and sustained at 21DPI. EphrinsA2 and -A5 were downregulated at 1DPI, returning to baseline levels by 21DPI. In contrast, adults revealed no expression of ephrin-A1 in control or post-ischemic brains. Acute and sustained upregulation of ephrinsA2 and -A5 were detected in adults. Antibody labeling revealed the expression of ephrinA1 (infants) and -A2/-A5 (adults) predominantly on astrocytes, with highest labeling intensity proximal to the infarct core, diminishing distally, directly. At both ages, upregulated ephrin ligands were co-expressed with EphA4. We confirmed the interaction of ephrinA1 and -A2/-A5 with the EphA4 receptor individually through EphA4 co-Immunoprecipitation (co-IP). In adults specifically, ephrinsA2 and -A5 were found to be co-expressed on RA with significant fluorescent puncta colocalization detected. Co-clustering of ephrinA2 and -A5 ligands was also detected through ephrin-A5 co-IP. Our data indicates that Eph/ephrin expression on RA after brain injury differs between infant and adults and provides greater evidence for a role in glia-glia communication after injury. Based on our current and previous results, we propose that ephrinA2/-A5 acts synergistically on EphA4 to promote astrocyte reactivity, proliferation and migration, resulting in more severe glial scarring in adult but not infant primates.

Disclosures: L. Teo: None. **J. Bourne:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

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Topic: C.08. Ischemia

Support: NIH PO1 HD 32573

NIH RO1 HL 66237

Title: iPS cells-derived astrocytes from subjects with Monge's disease are vulnerable to hypoxia/ischemia

Authors: *H. YAO[1], H. ZHAO[1], G. G. HADDAD[1,2,3]
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Abstract: The brain is one of the major target organs of Chronic mountain sickness (CMS) or Monge's disease, as manifested by the frequently seen CNS symptoms such as headache, dizziness, sleep disturbance, and mental confusion. Astrocytes play a critical role in this disease as they control fluid and electrolyte homeostasis. In this work, we obtained skin biopsies from CMS patients, healthy highlanders (non-CMS) and re-programmed the skin cells into induced pluoripotent stem cells (iPSCs). The iPSCs were then differentiated into neuroprogenitor cells and further into astrocytes. To compare the response of astrocytes to hypoxia/ischemia challenges between CMS and non-CMS, we conducted cell viability assay following chronic hypoxia (1% O_2), oxygen-glucose deprivation (OGD) and IS (a solution that mimics the ischemic penumbral environment) challenges. Cell viability was analyzed by propidium iodide stained cell counting which was normalized by the DAPI stained cell counting and presented as the percentage of injured cell number over the entire cell counting. Our results show that a 5 day chronic hypoxia induced a 19% (453/1949) cell death in CMS astrocytes, a significant increase over that in non-CMS (13%, 167/1169, χ^2 test, $p= 3.4x10^{-6}$). In OGD (24 hour) experiment, a 42% (675/1596) cell death was seen in CMS astrocytes, which doubles the amount of cell death seen in non-CMS astrocytes (19%, 206/1087, χ^2 test, $p=1.2x10^{-36}$). Finally, in IS (24 hour), cell death is 37% (593/1600) in CMS and 36% (1289/3600, χ^2 test, $p= 0.384$) in non-CMS astrocytes, respectively, with no signigicant difference between the two groups. In summary, compared with non-CMS astrocytes, CMS astrocytes show increased vulnerability to hypoxia/ischemia challenges and this may explain why pronounced CNS symptoms are seen in CMS patients.

Disclosures: H. Yao: None. **H. Zhao:** None. **G.G. Haddad:** None.

Poster

420. Ischemia: Cellular Mechanisms and Neuroprotection III

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 420.19/X1

Topic: C.08. Ischemia

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Phenotypic and transcriptomic astrocyte heterogeneity in the uninjured and post-stroke brain

Authors: *A. J. GLEICHMAN[1], M. V. SOFRONIEW[2], S. T. CARMICHAEL[3]
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Abstract: There is emerging evidence for distinct astrocyte phenotypes in the developing and adult CNS. In the adult brain, acute injury, such as in stroke, causes an initial process of local scar formation that confines the damage, and a later and limited process of tissue repair that involves the formation of new connections and new blood vessels. Astrocytes are central to both scar formation and tissue repair after stroke, but little is known about how the astrocyte mediates these dual functions of injury and repair. Here, we explore astrocyte phenotypes in the normal brain and after injury using viral vector-defined morphological analyses, immunohistochemical analyses of key molecular systems, and RNAseq of defined astrocyte populations *in vivo*, using both white matter and cortical stroke models. Astrocyte-specific lentiviral vectors driving fluorophore expression were delivered to distinct zones of astrocyte activation after stroke. The complete arbor of individual astrocytes was visualized, allowing quantification of the length and thickness of processes, arbor complexity, and domain size. These quantifications demonstrate the existence of multiple morphologically distinct astrocytic populations based on distance from infarct. These populations also differ in the expression of functionally relevant astrocytic proteins, including the inflammatory master regulator Lcn2, the water transporter Aquaporin-4, the proliferation marker Ki67, and the glutamate transporters GLT-1 and GLAST. Transcriptomic analyses of different astrocytic populations are being conducted using the RiboTag mouse model, in which actively translating mRNA can be isolated exclusively from Cre-expressing cells. By crossing the RiboTag line with a GFAP-Cre line, we have generated mice in which astrocytes express tagged ribosomes. Astrocytic mRNA was isolated from uninjured cortex vs white matter and analyzed via RNAseq; these data reveal differences in both gene and splice isoform expression patterns between uninjured fibrous vs protoplasmic astrocytes. Similar analyses are being conducted post-stroke, in morphologically and

phenotypically distinct subsets of astrocytes isolated via laser capture microscopy. Together, these data demonstrate functional differences between astrocytic subpopulations and suggest molecules and pathways to target in the development of astrocyte-directed approaches to promote neural repair post-stroke.

Disclosures: A.J. Gleichman: None. **M.V. Sofroniew:** None. **S.T. Carmichael:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.01/X2

Topic: C.10. Trauma

Support: NIH R03 NS077098

NIH R01 NS-065052

Phoenix Children's, Hospital Mission Support Funds

Science Foundation Graduate Research Fellowship Program (Ortiz)

College of Liberal Arts and Sciences at Arizona State University

Title: In the wake of diffuse traumatic brain injury, enduring dendritic hypertrophy within the basolateral amygdala

Authors: A. N. HOFFMAN[1,3,4], J. B. ORTIZ[1], T. C. THOMAS[5,6,7], J. LIFSHITZ[1,5,6,2], *C. D. CONRAD[1,2]
[1]Psychology, Arizona State Univ., TEMPE, AZ; [2]Neurosci. Program, Arizona State Univ., Tempe, AZ; [3]Psychology, [4]Neurosurg., UCLA, Los Angeles, CA; [5]Child Hlth., Univ. of Arizona Col. of Med., Phoenix, AZ; [6]Barrow Neurolog. Inst., Phoenix Children's Hosp., Phoenix, AZ; [7]Phoenix VA Healthcare Syst., Phoenix, AZ

Abstract: There is an emerging link between traumatic brain injury (TBI) and post traumatic stress disorder (PTSD), and the increasing numbers of veterans with TBI has created interest in their comorbidities. The amygdala, a phylogenetically conserved limbic structure, has long known to be involved in processing emotional and stressful stimuli, and is implicated in PTSD. Moreover, chronic stress alone results in dendritic restructuring in the amygdala, which persists

for up to one month after the stressor manipulation has terminated. Even a single stressor manipulation can produce long-lasting changes in amygdalar dendritic architecture. The amygdala has been largely overlooked in TBI research and reorganization in this structure may serve as a nidus for TBI-induced PTSD affective symptoms and a future target for treatment. In the current study, we hypothesize that enduring structural alterations within the basolateral amygdala (BLA) after TBI may be a mechanism underlying the TBI-PTSD comorbidity. The purpose of this study was to quantify temporal changes in dendritic complexity within the BLA following a single diffuse TBI using a midline fluid percussion injury model. Adult male Sprague-Dawley rats were subjected to a single moderate severity midline fluid percussion injury (1.9 atm; 6-10 min righting reflex), or sham surgery. At post injury days (PID) 7 and 28, sham and brain-injured rats were euthanized and brains were collected and processed for Golgi stain analysis (200μm sections; FD Rapid GolgiStainTM kit) to quantify dendritic complexity of individual BLA neurons. Fully stained, untruncated, and isolated pyramidal and stellate neurons were identified and reconstructed using a camera lucida microscope. The total number of branch points and overall dendritic length were quantified. Data from both pyramidal and stellate neurons were combined. Compared to sham, brain-injured rats at both PID 7 and 28 showed increased number of total branch points and overall branch length within the BLA. These data suggest a chronic enhancement of dendritic complexity within the BLA after a single TBI. Increased dendritic complexity would necessarily alter information processing into and through the extended amygdala, which may contribute a long-term risk factor or cause the development of PTSD after brain trauma. The amygdala remains a central focus for the continued analysis in the wake of TBI.

Disclosures: **A.N. Hoffman:** None. **J.B. Ortiz:** None. **T.C. Thomas:** None. **C.D. Conrad:** None. **J. Lifshitz:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

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Program#/Poster#: 421.02/X3

Topic: C.10. Trauma

Support: NIH Grant NS55012

Title: Time-sensitive molecular mechanisms underlying post-TBI remodeling of the inhibitory synaptic network

Authors: *P. N. LIZHNYAK[1], P. DE DOMENICO[2,1], A. K. OTTENS[1]
[1]Dept. of Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA; [2]Univ. of Messina, Messina, Italy

Abstract: Understanding the molecular processes that underlie the pathobiology of traumatic brain injury (TBI) is instrumental to developing more effective diagnostics and treatments. Of particular interest in our laboratory are the regenerative mechanisms initiated during a critical post-acute period following TBI. Omic analysis provides us with an unbiased means to discern the temporal progression of molecular events that fashion repair and reorganization. Using a controlled cortical impact model, we induced focal brain injury in male rats and collected tissues for proteomic and immunofluorescence microscopy between 2 and 14 days post-TBI. We employed a non-targeted, data-independent mass spectrometry method to quantify TBI-induced proteome dynamics within spared somatosensory cortex adjacent to the focal injury. In this study, we tested the hypothesis that an analogous molecular mechanism that initiates inhibitory network development would be activated during the post-acute repair period following TBI. We examined the peptide data related to inhibitory-specific isoforms of synaptic proteins to include synaptotagmin-2 (SYT2), neuroligin-2 (NL-2), gephyrin, and KCC2 to evaluate post-translational changes. Results demonstrate novel, temporally resolved changes at 2 and 4 days, respectively post injury, in ubiquitin and phosphorylative motifs of NL-2, a key initiator of inhibitory network remodeling. Coinciding at 4 days post-TBI, we resolved the reduction of KCC2 chloride transporter levels at the membrane, reverting inhibitory neurons to a development-like GABA-induced excitatory state. KCC2 level recovery then coincides with a transient de-polymerization of gephyrin at 7 days following injury, suggesting prominent plasticity of the inhibitory network. Immunofluorescence results affirm the temporal reduction in punctate NL-2 staining of inhibitory synapses as well as remodeling as suggested by Syt2 staining. Study findings reveal a temporal process by which the cortical inhibitory network is reorganized following TBI that reflects similar machinery employed during development. Further, these data define a transient critical period during post-acute recovery that represent a plausible boundary for therapeutic protection or enhancement of network repair as well as post-translational events by which to study the efficacy of novel pre-clinical interventions.

Disclosures: P.N. Lizhnyak: None. **A.K. Ottens:** None. **P. De Domenico:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.03/X4

Topic: C.10. Trauma

Support: La Marató TV3 (274/U/2011)

Title: Loss and recovery of olfactory function induced by excitotoxicity in rats as a model for secondary neuronal degeneration in Traumatic Brain Injury: Role of adult neurogenesis

Authors: *C. A. MARIN[1], I. TEJERO[2], I. ALOBID[3], J. BERENGUER[3], M. BERNABEU[4], S. CENTELLES[3], S. LAXE[4], E. LEHRER[3], F. MARIÑO-SÁNCHEZ[3], J. MULLOL[2]
[1]IDIBAPS NIF: Q-5856414G, 08036 Barcelona Cata, Spain; [2]IDIBAPS, Barcelona, Spain; [3]Hosp. Clin., Barcelona, Spain; [4]Inst. Guttmann, Barcelona, Spain

Abstract: Traumatic brain injury (TBI) constitutes one of the main causes of olfactory dysfunction. One event related to TBI is the secondary neuronal degeneration (SND), a downstream cascade of events promoting further damage. Excitotoxicity is a key factor in SND since during the TBI acute phase, a massive release of glutamate occurs. The role of excitotoxicity on TBI olfactory dysfunction is still unknown. The goal was to examine the olfactory dysfunction induced by bilateral administration of the glutamate agonist N-methyl-D-aspartate (NMDA) in the olfactory bulbs (OB) in the rat as an experimental model of SND. Sprague-Dawley rats were maintained in a food-deprivation schedule. Olfactory discrimination tests were performed before, 1 and 2 weeks after NMDA-lesion. The dish in which rats dug first and the spent time were recorded. NMDA or vehicle was bilaterally injected into OB (1, 2, or 3 injections of 1.5 µl). Nissl staining, NeuN, tyrosine hydroxylase (TH), and glial fibrillary acidic protein (GFAP) immunohistochemistry were performed in OB. Doublecortin, PSA-NCAM and PCNA immunohistochemistry were performed in the subventricular zone. One week after NMDA lesions, animals showed a significant 70% ($p<0.01$) decrease in correct trials when 3, but not 1 and 2, injections were administered ($p<0.01$). The time spent to achieve the correct odour increased ($p<0.05$). A recovery of olfactory function was observed two weeks after lesion ($p<0.01$). NMDA lesions resulted in neural injury through all bulb layers. The present results indicate that bilateral OB NMDA lesion is a useful tool to investigate excitotoxicity in the SND after TBI and to study the pathophysiology and repair mechanisms of the olfactory dysfunction. *This study was sponsored by a grant from La Marató TV3 (274/U/2011)*

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.04/X5

Topic: C.10. Trauma

Support: NH&MRC GNT1045125

Title: Regulation of newborn neuron survival and the inflammatory cell response after traumatic brain injury by suppressor of cytokine signalling 2 (SOCS2)

Authors: H. S. BASRAI, K. J. CHRISTIE, *A. M. TURNLEY
Anat. and Neurosci., The Univ. of Melbourne, Melbourne, Australia

Abstract: Suppressor of cytokine signalling-2 (SOCS2), a negative regulator of the JAK-STAT pathway, is expressed at the highest levels in the hippocampal CA3 region and dentate gyrus in the adult brain. We previously showed that SOCS2 overexpressing (SOCS2Tg) mice have increased survival of newly born adult hippocampal neurons. We have now examined hippocampal neurogenesis in SOCS2 knockout (SOCS2KO) mice. Adult SOCS2KO and wildtype mice were given one pulse of EdU per day for 7 days. One cohort was perfused on the 8th day to study effects on neurogenesis (neuroblast proliferation and differentiation) and a second cohort perfused at 35 days to examine newborn neuron survival. No difference in neurogenesis was observed between SOCS2KO and wildtype mice. However, SOCS2KO mice showed a reduction in the survival of newborn adult hippocampal neurons. This confirmed a role for endogenous SOCS2 in the regulation of newborn neuron survival in the hippocampus. Given that overexpression of SOCS2 enhanced basal adult neurogenesis, we hypothesised that SOCS2Tg mice may also display enhanced survival of newborn neurons following traumatic brain injury (TBI) compared to wildtype mice. Therefore, SOCS2Tg mice and littermate wildtype controls were subjected to a controlled cortical impact (CCI) or sham surgery. Mice were pulsed with EdU beginning immediately after injury once per day for 7 days to label cells proliferating in response to the injury. After behavioural analyses at 2, 7 and 33 days post-injury, brains were collected 35 days post injury to examine newborn neuron survival, glial and inflammatory cell responses. Newborn EdU+/NeuN+ neurons were identified in the injured cortex of animals of both genotypes following moderate but not mild CCI and SOCS2Tg mice showed functional improvement on a ladder test compared to wildtype mice. Further, there was a two fold increase in the total number of EdU+ cells in the injured cortex of SOCS2Tg animals compared to wildtype. CD11b+ microglia/macrophages were the major contributors to this increase. EdU+/Olig2+ cells were also present and their number was significantly, but not differentially, increased post-TBI in both genotypes. These results suggest a role for SOCS2 in modulating the inflammatory cell response after TBI, which may contribute to the functional recovery observed in SOCS2 overexpressing mice.

Disclosures: **H.S. Basrai:** None. **A.M. Turnley:** None. **K.J. Christie:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

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Program#/Poster#: 421.05/X6

Topic: C.10. Trauma

Support: Army Research Office W911NF-10-1-0276

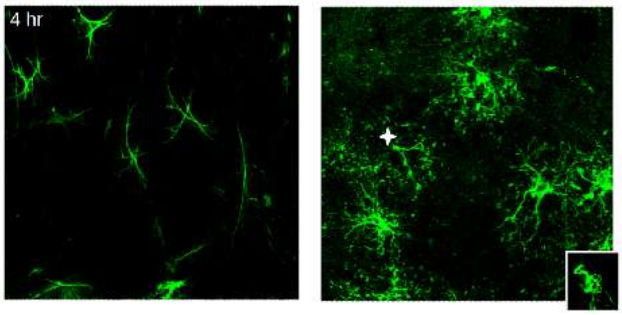
NF/SG VA Medical Center

Title: Simulated blast overpressure-induced astrocyte injury in an acute brain slice model

Authors: ***M. A. KING**[1,4], S. CANCHI[2], Y. HONG[2], J. FLINT[3], M. SARNTINORANONT[2], G. SUBHASH[2]
[2]Mechanical & Aerospace Engin., [3]Neurosci., [1]Univ. Florida, GAINESVILLE, FL; [4]DVA Med. Ctr., Gainesville, FL

Abstract: Exposure to explosive blasts can produce complex brain pathology and debilitating functional outcomes. The overpressure characteristics of the primary blast wave are considered to be potentially damaging to the brain. Astrocytes participate in neuronal metabolic maintenance, blood-brain barrier, regulation of the homeostatic environment, and tissue remodeling. Little is known about their specific susceptibility to blast injury apart from delayed reactive astrogliosis. Overpressure-induced tissue strain transients could mediate mechanical failure in astrocyte processes. Subsequent compromised function of single astrocytes would involve multiple neurons encompassed by the processes of individual astrocytes. Transient or sustained astrocyte injury could conceivably induce complex neuronal pathology sufficient to impair brain function, and be distributed among dispersed compartments of disturbed tissue too small to detect with diagnostic imaging. An *in vitro* rat brain tissue slice model was used to test whether direct interaction of tissue astrocytes with a simulated blast wave limited to overpressure alone will elicit acute, histologically detectable injury. A polymer split Hopkinson pressure bar (PSHPB) system was used to impart a single blast pressure peak to a slice chamber. Initial experiments control pressure at a level comparable to actual explosive blasts. Glial fibrilliary acidic protein (GFAP) immunofluorescence intensity and area fraction in confocal images were quantified and statistically analyzed by 2-way ANOVA (shock x time). Two acute astrocyte injury profiles have been identified during the initial hours after the overpressure: (a) astrogliosis

characterized by enhanced GFAP intensity without significant increase in tissue area fraction occupied by labeled astrocytes, and (b) clasmatodendrosis, an autophagic degradation of distal processes that has not been previously associated with blast-induced neurotrauma. The controlled testing platform provides insight on overpressure injury that is otherwise difficult to isolate.



Disclosures: **M.A. King:** None. **S. Canchi:** None. **Y. Hong:** None. **J. Flint:** None. **M. Sarntinoranont:** None. **G. Subhash:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.06/X7

Topic: C.10. Trauma

Support: NIH Grant NS056247

NIH Grant NS057758

Title: Matrix metalloproteinase-9 and osteopontin mediation of cellular response in the olfactory bulb during trauma-induced synaptogenesis

Authors: ***M. A. POWELL**, P. A. TRIMMER, T. M. REEVES, L. L. PHILLIPS
Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA

Abstract: Traumatic brain injury (TBI) causes damage to many different CNS circuits. This includes pathway disruption that can result in sensory deficits. Such deficits are attributed to TBI axotomy, which produces deafferentation and loss of synaptic organization. Olfactory receptor neurons are particularly vulnerable to axotomy, resulting in targeted deafferentation of synapses in the olfactory bulb (OB). Notably, this circuit can undergo reactive synaptogenesis, restoring

lost synaptic connections over time. Our laboratory has demonstrated that matrix metalloproteinases (MMPs) regulate TBI-induced synaptic repair. In OB, secreted gelatinase MMP-9 is acutely elevated after injury, consistent with its posited role in reshaping the local environment during synaptogenesis. Recently, the cytokine osteopontin (OPN) was identified as a MMP-9 substrate, its integrin binding domains exposed by MMP proteolysis. We hypothesize that injury-induced MMP-9/OPN interaction in the deafferented OB supports cell signaling and facilitates synapse recovery. Using FVB/NJ wild type (WT) and FVB MMP-9 knockout (KO) mice subjected to moderate central fluid percussion TBI, we analyzed OB OPN fragment generation at 3, 7, and 21d postinjury relative to paired sham-injured cases. These time points represent sub-acute degenerative and early regenerative phases of OB reactive synaptogenesis. MMP-9 activity of injured and control WT mice was also assessed by zymography. Results showed injury-induced elevation of MMP-9 activity at both 3d (34%) and 7d (4 fold), the latter statistically significant. By 21d, MMP-9 activity was not different from controls. Western blot OPN analysis revealed generation of a 47kD fragment, temporally correlated with changes in OB MMP-9 activity. KO of MMP-9 significantly attenuated expression of the 47kD OPN fragment at both 3 and 7d, suggesting that OB MMP-9 contributes to regulation of OPN signaling after TBI. To further explore MMP-9 and OPN expression in specific OB cell populations, we subjected mixed glial/neuronal cultures from WT and MMP-9 KO mice to OPN immunocytochemical analysis. Results showed limited OPN expression in WT cells. OPN did not co-localize with GFAP+ astrocytes or IBA1+ microglia, suggesting either neuronal or olfactory ensheathing cell labeling. Interestingly, MMP-9 KO OB cultures had a pronounced increase in OPN+ cells, as well as elevated glial density and OPN localization in microglial populations. Collectively, these data suggest that OB MMP-9 contributes to OPN processing after TBI, and that OPN is a likely mediator of postinjury cellular response during reactive synaptogenesis in the deafferented OB.

Disclosures: **M.A. Powell:** None. **P.A. Trimmer:** None. **T.M. Reeves:** None. **L.L. Phillips:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.07/X8

Topic: C.10. Trauma

Title: Mild traumatic brain injury (TBI) *in vitro*: Network activity changes and parameters of recovery

Authors: *D. SMITH[1], G. W. GROSS[2]
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Abstract: The complex pathology of TBI requires research on all levels: from studies of holistic brain injury to cellular and even synaptic disruption. In many cases, physical evidence does not exist, as standard CTI or MRI scans are not sensitive enough. Diffuse brain injury ranges from axonal damage after minor head injury to inflammatory responses and calpain-mediated cytoskeletal changes [1]. Although networks *in vitro* do not represent brain tissue *in situ*, the highly controlled environment and multifactorial readout provides quantitative injury data for establishing reliable damage thresholds, recovery profiles, and biochemical enhancement of recovery. We have combined a ballistic pendulum rapid acceleration device with multichannel recording from frontal cortex cultures. This allows comparisons of spontaneous network activity before, and within four minutes after injury at a variety of different impact forces with subsequent long-term (days to weeks) monitoring. Single 100-g impacts have shown hyperexcitation as well as activity decreases, frequently with full recovery to reference. However, multiple impacts of 100 g's have shown consistent, significant decreases in reference activity. Two to three sequential insults spaced by thirty to ninety minutes have shown 40-60% decreases in activity with no return to reference in 24 hrs (n= 8). Adhesion was not compromised, and major functional changes occurred without loss of active unit signatures. Rapid neuronal cell death seems to require higher g-forces, and observed early deficits may be of synaptic origin or microporation. Although exact impact forces for specific responses may differ between *in vitro* and *in situ*, the recovery from cellular damage as well as the efficacy of potential interventions can be determined *in vitro* and should be scalable to animal models. **[1] Povlishock JT, Becker DP, Cheng CLY, Vaughan GW (1983) Axonal Change in Minor Head Injury. J. Neuropath. & Exp. Neurol. 42(3): 225-242.**

Disclosures: D. Smith: None. **G.W. Gross:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: Center for Neuroscience and Regenerative Medicine (CNRM) career development fellowship to MKJ (Grant G1708X)

CNRM grant (G1703O) to ZG

Title: 2-photon in-vivo imaging of impaired balance between excitation/inhibition and acute vascular trauma in sensory barrel cortex correlates with growth of microinfarcts following open head CCI injury in mice

Authors: *M. K. JAISWAL[1,2], F. W. LISCHKA, Ph.D[1,2], X. XU[2], Z. GALDZICKI, Ph.D[1,2]
[1]Ctr. for Neurosci. and Regenerative Med., Bethesda, MD; [2]Dept. of Anatomy, Physiol. and Genet., USUHS, Sch. of Med., Bethesda, MD

Abstract: The adult brain is soft-wired and must undergo plasticity to support adaptation to an altered environment or injury. In response to sensory deprivation caused by TBI, the functional topography of the neocortex is altered such that cortical areas or 'maps' of deprived sensory inputs shrink, while maps of the remaining spared inputs expand. However, it is not known how sensory-driven activity in individual L2/3 neurons changes over time after brain injury, and how these changes differentially occur within local excitatory/inhibitory (E/I) neuronal populations in response to alterations in plasticity after injury. There are three central unanswered questions relating to alterations in plasticity after injury. First: What is the time window for the onset of neuronal deficits? Second: How do sensory and motor circuit remodeling change over the course of the recovery period? Third: To what extent does neuronal injury cause additional vasculature disruption, and vice versa? To address the first two questions, we have proposed using in-vivo imaging of neuronal [Ca2+] by two-photon microscopy to monitor the activity of E/I neurons in GAD67-GFP knock-in mice and assess the loss/gain of spontaneously evoked neuronal activity in the core of the microinfarct after TBI and sham surgery in acute and chronic TBI. In addition we assessed the loss or disruption of functionally evoked neuronal population activity in the core of the microinfarct by imaging in the relatively large hindlimb region of the primary somatosensory cortex, where peripheral electrical stimulation could reliably activate a large number of neurons. To address the last question, we monitored vascular changes caused by damage to the integrity of the BBB with dye specific for blood vessels/arteries with simultaneous recordings of Ca2+ transients in E/I neurons. 2-photon in-vivo Ca2+ imaging revealed a decrease in overall spontaneous activity within the neuronal population of TBI mice, due to a higher number of hypoactive excitatory/inhibitory L2/3 neurons within microinfarcts of injury epicenter. At the same time preliminary experiments revealed both excitatory and inhibitory neurons from TBI mice displayed an unexpected increase in their frequency of spontaneous Ca2+ transients in comparison to sham littermates. The number of excitatory neurons responsive to hindlimb stimulation appears to decrease precipitously in the injured post injury. Overall, our preliminary results suggest reorganization of cortical population activity, traumatized vasculature and impaired balance between E/I sensory circuits with vascular defects exacerbating neuronal activities.

Disclosures: **M.K. Jaiswal:** None. **F.W. Lischka:** None. **X. Xu:** None. **Z. Galdzicki:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.09/X10

Topic: C.10. Trauma

Support: NIH Grant NS077675

Title: Increased potassium current after mild traumatic brain injury

Authors: J. SUN[1], A. HANELL[1], *K. M. JACOBS[2]
[1]Anat. & Neurobio., Virginia Commonwealth Univ., Richmond, VA; [2]Virginia Commonwealth Univ., RICHMOND, VA

Abstract: Mild traumatic brain injury (mTBI) causes cognitive deficits and memory impairment in patients. Molecular pathological changes after mTBI, especially in diffuse axonal injury (DAI) are well known, but neurophysiological consequences are still emerging. Using the YFP-h mouse, that allows identification of both intact and axotomized layer V pyramidal neurons prior to whole cell patch clamp recording, we have previously reported that intrinsic neuronal properties, including action potential amplitude, were altered at one and two day survival time points. Potassium channels play a critical role in shaping action potentials and modulating intrinsic neuronal membrane excitability. The present study investigates A-type (IA) and delayed rectifier (Ikd) potassium currents in this model, that employs a mild central fluid percussion injury (cFPI) without contusion or cell death. Whole cell patch clamp recordings were made from YFP+ layer V pyramidal neurons within somatosensory cortex. Total Kv current (IK) was recorded in voltage clamp using a 300 ms prepulse at -110 mV, followed by 500 ms voltage steps (by 10 mV) from -80 mV to +70 mV. Ikd was recorded by a 300 ms prepulse at -10 mV (to inactivate IA), followed by 500 ms voltage steps (by 10 mV) from -80 mV to +70 mV. IA was isolated by subtracting Ikd from total IK. For all groups, $n \geq 11$ cells. The whole-cell current and current density of IA was increased in both intact (current 58%, density 84%) and axotomized (current 74%, density 98%) neurons one day after cFPI compared to sham and naïve controls (t-test. $p<0.05$). At the two day survival time point, in axotomized neurons IA was significantly reduced compared to 1 day axotomized, but still significantly increased compared to control (current 54%, density 97%, t-tests, $p<0.05$). In intact cells at two days, IA was not significantly different from controls (t-test, $p>0.05$). Examination of Ikd showed no alteration for either axotomized or intact neurons at one or two days post cFPI. The kinetics of voltage dependent

activation and steady-state inactivation of IA and Ikd were also unchanged after cFPI. The increased IA current may reflect compensatory changes since the groups affected, are the same ones that showed increased intrinsic excitability (Greer et al 2012 J Neurosci, 32:6682; axotomized at one and two days and intact at one day survival). The lack of decreases in either IA or Ikd currents suggest that additional alterations in either sodium or other potassium channels must underlie the previously observed increased action potential amplitude and decreased after-hyperpolarization duration. Supported by NIH grant NS077675.

Disclosures: J. Sun: None. **K.M. Jacobs:** None. **A. Hanell:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.10/X11

Topic: C.10. Trauma

Title: Blockade of the N-type voltage gated calcium channel reduces intracellular calcium accumulation following injury visualized using the gene-encoded calcium sensor GCaMP6

Authors: *S. HUANG[1], G. G. GURKOFF[2], R. J. GARAJEHDAGHI[1], L. TIAN[3], B. G. LYETH[2], R. F. BERMAN[2]
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Abstract: An estimated 3.8 million Americans experience traumatic brain injury (TBI) annually, and over 5.3 million individuals report chronic deficits related to TBI. Unfortunately there are no pharmacological treatments that substantially improve neurological outcome after TBI. Developing effective therapies requires a thorough understanding of the cellular and molecular events that lead to cell dysfunction and death following TBI. One of the early and critical mechanisms thought to trigger secondary injury is the rapid accumulation of intracellular calcium ($[Ca^{2+}]_i$) after TBI, suggesting that prevention of this event could reduce cell injury and cell death. In the past it has been difficult to examine the time course of changes in $[Ca^{2+}]_i$ that occur over hours to days following trauma longitudinally in the same neuron. In the present study we utilized a gene encoded calcium indicator to successfully image $[Ca^{2+}]_i$ levels and signaling in the same neurons over time. Specifically, mixed neuronal/astrocyte co-cultures were grown and infected with GCaMP6 using an adenoviral vector. Fluorescence imaging in GCaMP6-infected neurons showed that the majority of neurons were expressing the construct

and allowed for longitudinal imaging of intracellular calcium and synaptic activity in neurons before and after mechanical injury. Compared to baseline, injured neurons showed a large (i.e., >400%) increase in intracellular calcium that returned to baseline within 24 hr after injury. Pre-treatment with the N-type voltage gated calcium channel antagonist SNX-185 reduced the rise in intracellular calcium, validating our previous findings with SNX-185 based on calcium imaging with Fura-2-AM. These results establish the usefulness of gene encoded calcium indicators for imaging cells longitudinally in an *in vitro* model of traumatic injury and replicate our earlier findings of elevated intracellular calcium using the same *in vitro* TBI model. Furthermore, these data demonstrate that blockage of N-type voltage gated calcium channels reduces the pathological increase in intracellular calcium and represents a potential therapeutic target for neuroprotection in TBI. Therefore, utilization of gene encoded calcium indicators has the potential to improve our understanding of mechanisms related to primary and secondary neuronal injury and also to guide the development of future neuroprotective strategies.

Disclosures: S. Huang: None. **G.G. Gurkoff:** None. **R.J. Garajehdaghi:** None. **L. Tian:** None. **B.G. Lyeth:** None. **R.F. Berman:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 421.11/X12

Topic: C.10. Trauma

Support: CHRB Grant 236-040-12

Title: Injury-induced electric fields drive astrocyte reactivity *in vitro*

Authors: *M. L. BAER, S. C. HENDERSON, R. J. COLELLO
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Abstract: Traumatic injury to the central nervous system (CNS) induces reactivity in surrounding astrocytes that can have both positive and negative influences on repair. Astrocyte reactivity is characterized by hypertrophy of the soma; realignment of cellular processes; up-regulation of the cytoskeletal elements glial fibrillary acidic protein (GFAP), vimentin, and nestin; proliferation; and migration to the lesion border. While reactive astrocytes play a necessary role in the injury response, they ultimately inhibit regeneration by blocking sprouting axons. Altering this reactive phenotype could facilitate regeneration by selectively enhancing

growth-promoting behaviors while attenuating inhibitory ones. However, it is unclear what physiologic change at the injury site induces the many cellular behaviors characteristic of astrocyte reactivity. Injury currents that induce a 10-fold increase in the physiologic electric fields (EF) have been measured in mammalian skin, cornea, and bone where they have been shown to direct cellular behaviors essential to the reparative responses in those tissues. A similar increase in physiologic EFs has been reported in the mammalian CNS upon injury, but the extent to which these injury-induced EFs can drive astrocyte reactivity has not been fully elucidated. With this in mind, our lab has become interested in chronicling the injury currents in the mammalian CNS following a stab injury, and in determining whether the intensity and duration of these EFs is sufficient to drive the reactive phenotype. Using a vibrating probe electrode to measure the injury currents, we found that a stab wound in the rat cortex induces an injury current similar to those demonstrated in other injured mammalian tissues. We then demonstrated that these physiologic EFs drive multiple behaviors in isolated astrocytes *in vitro* that are characteristic of the reactive phenotype see *in vivo*. EF exposure induces hypertrophy of the cell soma with increased expression of the cytoskeletal elements GFAP, vimentin, and nestin, as well as other proteins associated with reactivity. We found that EFs induce a marked increase in proliferation that peaks 48 hours after onset of exposure. Furthermore, EFs affect astrocyte migration, directing them toward the anode as early as 3 hours after field onset. Together, these unique observations demonstrate that the intensity and duration of the physiologic EFs found after injury *in vivo* are capable of driving reactive behaviors of astrocytes *in vitro*. This suggests that injury-induced EFs may be an important stimulus of reactive astrocytosis and represents an ideal target to induce a more regenerative response.

Disclosures: **M.L. Baer:** None. **S.C. Henderson:** None. **R.J. Colello:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Title: Post-traumatic switch in constitutive toll-like receptor 4 modulation of dentate excitability

Authors: *A. A. KORGAONKAR, Y. LI, V. SANTHAKUMAR
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Abstract: Brain injury a leading cause of acquired epilepsy and neurological disability in young adults, leads to early activation of immune responses and cellular and synaptic changes in the hippocampal dentate gyrus. We find a post-injury increase in dentate hilar neuronal expression of toll-like receptor4 (TLR4), a class of innate immune receptors implicated in enhanced hippocampal excitability in epilepsy. Here we examine if activation of TLR4 signaling after brain injury modulates dentate excitability *in vitro* and seizure thresholds *in vivo*. Wistar rats (25-27 day old) were subject to moderate (2 atm) lateral fluid percussion injury (FPI) or used as sham-controls (Gupta et al., 2012). The effect of TLR4 ligands (agonists, LPS-RS and TLR4 antibody and agonist, HMGB1) on perforant-path evoked granule cell population spike amplitude was examined in hippocampal slices 3 and 7 days FPI. A cohort of rats were administered LPS-RS (5ug) or vehicle (bolus hippocampal injection) 24 hrs after FPI and tested for changes in latency to kainic acid (5mg/kg, i.p.) induced seizures 30 days after FPI. In slices from control rats, TLR4 antagonists consistently increased (anti-TLR4: 19.35±51.45%) while HMGB1 suppressed (-60.91±13.42%) afferent evoked dentate population spike amplitude. Unlike controls, TLR4 antagonists decreased (anti-TLR4: -79.0±18.8%) dentate population spike amplitude after FPI indicating that TLR4 signaling contributes to the post-traumatic increase in dentate excitability. HMGB1 increased dentate excitability after FPI (83.77±14.36%) revealing a post-injury reversal of TLR4 effect on network excitability. Consistent with *in vitro* studies, LPS-RS treatment reduced the latency for kainic acid induced seizures in sham-controls and significantly prolonged seizure latency after FPI. Together, our *in vitro* and *in vivo* data demonstrate a constitutive effect of TLR4 signaling on dentate excitability. We identify a post-traumatic reversal in the direction of TLR4 modulation of excitability which contributes to early dentate hyperexcitability after brain injury. A single post-injury treatment with a TLR4 antagonist has a potential to prophylactically prevent lasting decreases in seizure threshold after brain injury. However, the ability of TLR4 antagonists to reduce seizure thresholds in controls needs to be considered while targeting TLR4 for therapy. Moreover, the mechanisms and physiological role of the novel constitutive and bi-directional effects of TLR4on dentate excitability, identified here, need further investigation.

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Loyola University Neuroscience Institute

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Title: The effect of repeated binge alcohol combined with TBI on the subventricular zone microenvironment

Authors: *S. T. TON[1], I. C. VAAGENES[2], S.-Y. TSAI[2], D. J. SHEPHERD[1], V. A. HUSAK[2], D. C. NOCKELS[1], G. L. KARTJE[1,2]
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Abstract: We have previously found that a repeated dose of binge alcohol prior to traumatic brain injury (TBI) leads to worse recovery on a sensitive test of skilled forelimb function (Vaagenes et al., 2014). One means by which the brain may compensate for injury is in the mobilization of neural precursor cells. We therefore sought to determine the effect of binge alcohol at the time of TBI on subventricular zone (SVZ) and perilesional neural precursor cells. Adult, male rats were given daily doses of alcohol, either by injection (2gm/kg/i.p/day) or oral gavage (3gm/kg/day) for three consecutive days. One hour after the final dose, animals were given a TBI directed to the forelimb sensorimotor cortical area and sacrificed three weeks later. Brains were immunostained for proliferating cell nuclear antigen (PCNA) and doublecortin (DCX) to label neural precursor cells. We found that this short three day repeated dose of binge alcohol prior to TBI reduced the proliferation of neural precursor cells in the SVZ and perilesional area as well as decreased the differentiation of these cells into neurons. Furthermore, we found that the number of activated microglia as measured by ED-1 immunohistochemistry was reduced in the SVZ of TBI rats treated with binge alcohol compare to TBI alone. In addition to important immune functions, microglia also provide a supportive growth environment in the

SVZ through the release of various trophic factors. Neurotrophic factors play various roles in the regulation of neurogenesis after TBI; for instance, epidermal growth factor (EGF) and fibroblast growth factor (FGF) promote proliferation of neural stem cells while brain derived neurotrophic factor (BDNF) promotes survival of newborn neurons (Richardson et al., 2010). We are currently investigating changes in neurotrophic factor expression and signaling after binge alcohol and TBI in the SVZ microenvironment, and how this relates to recovery after brain damage.

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Topic: C.10. Trauma

Support: CNRM

NMSS

Title: Heterogeneous TBI models reveal differential effects in the SVZ and divergent sonic hedgehog (Shh) signaling pathways in neuronal and oligodendroglial progenitors

Authors: A. J. MIERZWA[1,2], G. M. SULLIVAN[1,2], L. A. BEER[1,2], S. AHN[3], *R. C. ARMSTRONG[1,2]
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Abstract: The regenerative capacity of the CNS must be optimized to promote repair following traumatic brain injury (TBI) and may differ with the site and form of damage. In the adult CNS, Sonic hedgehog (Shh) maintains neural stem cells and promotes oligodendrogenesis. We examined whether Shh signaling contributes to neuronal (DCX) or oligodendroglial (NG2) progenitor responses to two distinct TBI models. Gli1 transcriptional activation indicates high levels of Shh signaling through the Smoothened receptor in the canonical pathway. Shh-responsive cells were heritably labeled *in vivo* using Gli1-CreERT2;R26-YFP bitransgenic mice with tamoxifen administration on days 2 and 3 post-TBI. All injuries were at the coronal level of

bregma to evaluate the response from cells in the subventricular zone (SVZ), the largest germinal zone in the adult mammalian brain. Mice were examined at 2 and 6 weeks post-TBI. In the first TBI model, injury to the cerebral cortex was produced with mild controlled cortical impact onto the overlying dura mater. YFP cells decreased in cortical lesions. Total YFP and YFP cells double-labeled with DCX increased in the SVZ, indicating Shh pathway activation in neural stem cells and neuroblasts. The alternate TBI model of impact onto the skull at bregma produced traumatic axonal injury in the rostral corpus callosum. YFP cells within the SVZ decreased at 2 weeks and then normalized by 6 weeks. In the corpus callosum, YFP cells were extremely rare, even after injury. YFP labeling was rarely found in NG2 progenitors, indicating a lack of Gli1 transcriptional activation in oligodendrocyte lineage cells. NG2 progenitors increased in the cortex, with a similar trend in the corpus callosum. To further test for Gli1 activation potential, Smoothened agonist (SAG) was microinjected into the corpus callosum to directly activate Shh signaling. YFP cells and NG2 progenitors increased in the SVZ but were not double-labeled, indicating an *in vivo* effect of Smoothened signaling without Gli1 activation in NG2 progenitors. Therefore, in all conditions, neuroblasts exhibited differential Shh pathway utilization compared to oligodendroglial progenitors. Furthermore, cortical versus white matter damage from TBI produced opposite responses of Shh-activated neural stem/progenitor cells within the SVZ. This work was supported by the Department of Defense within the Center for Neuroscience and Regenerative Medicine and by the National Multiple Sclerosis Society.

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Title: Heme Oxygenase 1 and Lipocalin 2 as potential modulators of vascular disruption after traumatic brain injury

Authors: *N. H. RUSSELL[1], L. L. PHILLIPS[2]
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Abstract: Heme Oxygenase 1 (HO-1), the inducible form of Heme Oxygenase, degrades heme into biliverdin, CO, and iron. It is a heat shock protein, robustly induced by CNS vascular hemorrhage following traumatic brain injury (TBI). Notably, HO-1 releases highly oxidative iron, which can promote local pathology, as well as up-regulate transcription, potentially affecting neural plasticity during postinjury recovery. Lipocalin 2 (LCN2) is a scavenging/trafficking siderocalin, which traps bound iron and relocates it to intracellular functional sites. LCN2 also persistently binds and activates matrix metalloproteinase-9 (MMP-9), a secreted gelatinase which affects local injury/repair mechanisms. Microarray analysis revealed significant elevation of HO-1 and LCN2 mRNA in rat cortex and hippocampus after fluid percussion TBI, suggesting that HO-1 and LCN2 act in concert to influence recovery within these brain regions. We hypothesized that trauma-induced neuroplasticity at sites of vascular damage is mediated by a pathway involving focal HO-1 expression and iron generation, which then drives LCN2 induction and MMP-9 activation. Twenty four hours after moderate central fluid percussion TBI, HO-1 protein expression in cortex and hippocampus was documented by Western blot (Wb) and immunohistochemistry (IHC). In parallel imaging experiments, HO-1 and LCN2 were examined for co-expression. Wb analysis revealed that diffuse TBI elevated HO-1 by 153% in hippocampus, and 455% in cortex relative to sham injured controls, all statistically significant effects. Wide field immunofluorescence microscopy showed HO-1 elevation in white matter tracts and at the injury site, both areas known to exhibit hemorrhage and neurodegeneration after injury. Interestingly, HO-1 was also elevated in lateral cortex and hippocampal subsectors, regions not associated with hemorrhagic bleeds. Confocal imaging demonstrated primary HO-1 localization within a subset of GFAP positive astrocytes at 24 hr post injury, in both the lateral cortex and hippocampus. HO-1 was also localized within IBA1 positive microglia surrounding gross hemorrhage and necrosis. LCN2 IHC experiments in the same tissue revealed LCN2 and HO-1 co-expression in the same subset of reactive astrocytes. These results show that TBI induces focal HO-1 up-regulation at hemorrhagic sites and in regions without overt vascular disruption. This HO-1 response involves both astrocytes and microglia, and is correlated with local LCN2 increase. Ongoing mapping of postinjury HO-1 and LCN2 response will determine their potential molecular interaction during recovery and role in MMP regulation.

Disclosures: N.H. Russell: None. **L.L. Phillips:** None.

Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Title: Deep brain stimulation: underlying neural mechanisms for recovery from traumatic brain injury

Authors: *H. KATNANI, J. ARONSON, M. THOMBS, E. ESKANDAR
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Abstract: In a previous publication (Katnani et al.; Society for Neuroscience Abstracts 2013) we described the enhanced functional recovery of mice in a sub-acute phase of traumatic brain injury (TBI) as a result of striatal stimulation. We found that brain injured mice receiving precisely timed stimulation performed significantly better then sham mice on a spatial memory task and also reached the same performance level of uninjured mice. In a follow-up study, we investigated potential neural mechanisms facilitated by stimulation that could underlie improved behavioral performance. Here we present results from an immunohistochemistry analysis, which first utilized c-fos expression to elucidate activated pathways, and second utilized specific neural markers for expression of neurogenesis and neuroplasticity in activated brain regions. In addition, whole transcriptome analysis was employed to broadly evaluate up-regulated genes caused by striatal stimulation in key regions associated with spatial memory such as the hippocampus, ventral striatum and prefrontal cortex. Evidence suggests that striatal stimulation can promote and cause proliferation in the brain, with neural proliferation observed in the subventricular zone. Furthermore, we speculate that these mechanisms facilitate the necessary neuroplastic changes to compensate for injured brain regions, thus allowing mice to overcome impairments at a faster rate and to a greater magnitude.

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Topic: C.10. Trauma

Title: Recovery of serotonin axons following a neocortical stab injury

Authors: *S. E. DOUGHERTY, D. J. LINDEN
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Abstract: Axon degeneration is a common occurrence in neurological disorders such as stroke and traumatic brain injury. While most of this axonal loss is as yet irreversible, some neuron populations have shown a propensity to recover their axonal arbors through regeneration (of injured axons) or compensatory sprouting (of spared axons). Serotonergic neurons display an enhanced capacity for axonal regeneration after acute injury. One potential explanation is the ability of serotonergic axons to survive and grow within hostile extracellular conditions. Previous studies have shown that serotonin axons can extend through otherwise nonpermissive environments including glial scars and within the subventricular zone. To assess serotonin axon degeneration, regeneration, and sprouting following acute brain injury, we used a neocortical stab model in a Slc6A4 (serotonin transporter) - soluble EGFP BAC transgenic mouse. One week after a cortical stab injury we are able to observe axonal damage and astroglial activation. There is a marked increase in GFAP staining surrounding the injury site throughout the cortical layers. This is concurrent with the appearance of bulbous fractured GFP staining suggesting the presence of degenerating serotonergic axons. At 10 weeks post-injury we observe an increase in axonal EGFP staining, including contiguous fibers extending across the damaged area, as well as a reduction in glial scar associated markers. In order to evaluate the dynamics of serotonergic axonal sprouting and regeneration, we are performing chronic *in vivo* two-photon imaging with a cranial window overlying the neocortex. These studies will reveal the dynamic nature of serotonin axonal regeneration and sprouting following a glial scar-forming injury and will provide insight into the process through which serotonergic axons overcome otherwise nonpermissive growth conditions.

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Poster

421. Traumatic Brain Injury: Neurogenesis and Neurophysiology

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Topic: C.10. Trauma

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NS069629

Title: The role of estrous stage cycle on synaptic transmission and behavior in female mice following mild traumatic brain injury

Authors: *K. A. FOLWEILER[1,2], C. CRUZ[2], A. S. COHEN[2,1]
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Abstract: Traumatic brain injury (TBI) affects over 1.5 million people in the United States each year with as many as 75% of cases considered mild TBI (mTBI) (Faul et al. 2010, Sosin, Sniezek and Thurman 1996). mTBI has been shown to lead to a wide array of long-lasting cognitive impairments including changes in working memory deficits. Despite prevalence and growing public concern, currently no effective therapies or treatments exist to attenuate neurological deficits resulting from mTBI. Females account for a significant portion of affected individuals; however, it is unknown whether recovery from injury is influenced by the levels of circulating ovarian steroid hormones (e.g., estrogens, progesterones) present in the brain depending on the menstrual cycle stage at time of insult. Therefore, the goal of this study was to assess the effects of female estrous cycle stage—the mouse correlate of the human menstrual cycle—on alterations in synaptic transmission and behavioral phenotype following mTBI. In order to model mTBI in mice we employed lateral fluid percussion injury (LFPI). LFPI is a commonly used rodent model of brain injury that reproduces many key features of human TBI including neuronal cell loss, gliosis, ionic perturbation and memory deficits (Dixon et al., 1987, McIntosh, 1987, 1989, Smith et al., 1991). At time of injury, mouse estrous cycle stages—estrus, diestrus, metaestrus, proestrus—were recorded. Six days following LFPI, we investigated changes in net synaptic efficacy in the hippocampus. We found a reduction in the Input/Output curves similar to that previously reported in brain injured male mice. That is, a decrease in the slopes of field excitatory postsynaptic potentials recorded in stratum radiatum of area CA1 were reduced in brain-injured mice across all cycle stages except for the estrous stage, which resembled the fEPSP slopes of sham controls**.** Furthermore, preliminary data from an elevated plus maze assessment suggests that there is little apparent injury effect on anxiety-like behavior in any of the cycle stages with the exception of the estrous stage (i.e., mice injured in estrous stage spent less time in the open arms of the maze compared to their sham counterparts). While it is unclear if the physiological alterations seen in hippocampal area CA1 are involved in observed behavioral changes, these results overall indicate that estrous cycle stage has an effect on synaptic transmission and behavioral outcome following mTBI.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Support: National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013R1A1A2013440)

Title: Changes in temporal and regional protein expression of peroxisome proliferator activated receptors (PPARs) after thoracic spinal contusive injury in rats

Authors: J.-H. OH, *Y. KIM, Y. YOON
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Abstract: Spinal cord injury leads to abnormal sensation and motor deficit below the level of lesion site that is caused by loss of myelination and cavity formation in the injured epicenter. Spinal cord is lipid-rich tissue owing to myelin sheath. Thus, lipid metabolism is important to regulate functions of the spinal cord. After spinal cord injury, inflammatory mediators such as eicosanoids are released and act as peroxisome proliferators that activate peroxisome proliferator activated receptors (PPARs), which play a key role in lipid metabolism and cellular differentiation. PPARs are classified into three isoforms (α, β/δ, γ) in the spinal cord. However, changing of their protein expression in the spinal cord after traumatic injury has not been demonstrated. Male Sprague-Dawley rats were anesthetized with ketamine/rompun mixture (1:4). Ten grams weight dropped from 6.25, 12.5 and 50 mm height onto the spinal cord of the 10^{th} thoracic vertebra level in rats after laminectomy using NYU device. We examined protein expression of PPAR-α, β/δ and γ in rostral, caudal, injured epicenter and L4-5 of the spinal cord with time after injury (6, 12, 24h, 3d, 1, 3 and 5 weeks). Patterns of PPAR-γ expression were similar among all of injured groups (6.25, 12.5 and 50 mm groups). PPAR-γ expressions were excessively increased from 6 hours and were maintained until 3 days after injury. After then, those were gradually returned to basal level. Expression of PPAR-γ was the greatest in the epicenter. Changing of PPAR-γ expression was spread around region, especially caudal direction that related to injury severity. Patterns of PPAR- β/δ expression were similar in both 6.25 and 12.5 mm groups, but this was appeared different pattern in 50 mm group. Expression of PPAR-β/δ in the caudal region was increased from 6 hours and was gradually increased over time after injury. In epicenter and rostral region, those were appeared from 3 days after injury. PPAR-α expression was increased until 3 days and was decreased from 1 week after injury at epicenter in a 6.25 mm group, while this was gradually decreased from 6 hours in a 50 mm group. However,

PPAR-α expression was not appeared relevant to difference of region, and was almost not changed at L4-5 region in all of injured groups. These results suggest that PPAR-γ is associated with pathophysiological mechanisms in the early phase, and PPAR-β/δ is associated with those mechanisms in the late phase after spinal cord injury. Expression of PPAR-γ and PPAR- β/δ seems to be related to the severity of injury. The present data serve as a reference for further study, which modulates neuroinflammation or myelination after spinal cord injury.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Title: Leveraging the VISION-SCI database to identify conserved features of forelimb function across species

Authors: *J. L. NIELSON[1], E. S. ROSENZWEIG[2], E. SALEGIO[1], K. D. ANDERSON[3], R. R. ROY[4], G. COURTINE[5], V. R. EDGERTON[4], O. STEWARD[6], M. H. TUSZYNSKI[2,7], M. S. BEATTIE[1], J. C. BRESNAHAN[1], A. R. FERGUSON[1]
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Lausanne, Switzerland; [6]Reeve-Irvine Res. Ctr., Univ. of California Irvine, Irvine, CA; [7]VAMC, La Jolla, CA

Abstract: Multiple efforts are currently underway to promote regeneration of long tract axons following spinal cord injury (SCI), specifically the corticospinal tract (CST). To understand the translational potential of emerging therapies for CST regeneration, parallel studies in small and large animal models are necessary. Here, we perform large-scale data-driven analytics coupled with stereological analysis of CST fiber density in rat and monkey SCI models to help identify multivariate features of CST-related functional recovery and assess species-specificity of these effects. Datasets for previously published studies in rats (Rattus norvegicus, n=13) and monkeys (Macaca mulatta, n=10) with cervical SCI and CST labeling were extracted from the Visualized Syndromic Information and Outcomes for Neurotrauma-SCI (VISION-SCI) database. Rats received graded bilateral cervical SCI (Infinite Horizon impactor severity 200 or 250 kilodyne) at cervical level 5-8 (C5-C8), and were tested for forelimb locomotion, gripping function; tissue sparing and CST fibers were quantified. Monkeys received unilateral hemisections at C7-C8, followed by monitoring for recovery in the activity chair and open-field, and quantification of tissue sparing and CST fibers. Stereological quantification was used to count labeled CST fibers in the spinal cord in both species. Syndromic analysis was performed using principal component analysis (PCA) to map species-specific multivariate patterns of functional recovery and CST sparing. A standardized dataset was created to combine matched measures (e.g. locomotion, gripping) and a separate PCA was performed to identify conserved patterns that may translate between species. Preliminary findings suggest the CST in rats contributes more to gripping function than general locomotion, whereas the CST in monkeys is involved in multiple skilled reaching, gripping and exercise cage tasks. A conserved syndromic measure was identified between rats and monkeys that were sensitive to injury, but not the effect of species. These results provide a proof-of-concept illustration that data-driven multivariate analytics can help identify translational outcome features to accelerate comparisons across species. However additional subjects, outcome measures, and injury paradigms in both species need to be assessed for cross-validation of these preliminary patterns.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Magnetic spinal cord pulsation-cancelation injection system for region-specific vector and cell delivery: A preclinical study in naïve and spinally-injured minipigs

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Abstract: Background: Development of a simple, safe and easily accessible spinal injection system is critical for a successful transition of cell-replacement-based and gene therapy-based therapies into clinical practice. One of the critical limitations in performing a safe spinal cell/vector delivery is our ability to effectively eliminate a spinal cord pulsation during spinal injections, thus increasing the risk of secondary spinal cord injury. Here we characterize a safety profile of spinal magnetic injection system which is simple to use, can be effectively coupled with currently existing XYZ manipulators and effectively eliminates spinal cord pulsation effect during spinal injection. Methods: A spinal magnetic injection system was constructed by utilizing the 30G injection needle anchored between two expulsing magnetic field, thus permitting the generation of a pulsation-cancellation effect in spinally placed needle. The safety of the injection system was tested in : i) naïve non-injured minipigs and, ii) in previously L3 spinal segment-injured minipigs at 2 months after injury. After dorsal L2-L3 laminectomy animals (N=3 in each group) received spinal parenchymal injection of human embryonic stem cell derived neural precursors or human fetal spinal cord derived stem cells. After cell grafting animals survived for 4-6 weeks while being continuously immunosuppressed with Prograf (0.025/kg/12hrs). The presence of grafted cell was validated by immunofluorescence staining of transverse spinal cord sections with human-specific (hNUMA, HO14, hNSE, hSYN) and non-specific (DCX, MAP2, Chat) antibodies and analyzed with confocal microscopy. Results: In all animals a consistent presence of grafted cells in the targeted central gray matter was seen. Individual grafts were readily identified by using human-specific nuclear (hNUMA) staining. At 6 weeks after grafting an extensive axo-dendritic sprouting was seen in segments rostral and caudal the cell injection core. No injection-related injury (such as necrotic cavities, tissue displacement) was seen in any animal. A comparable cell engraftment was seen in both naïve and L3-segment-injured animals. Conclusion: These data indicate that this spinal injection system can effectively and safely be used for spinal delivery of cells or any injectable substances (vectors, drugs) in large animals and in human.

Disclosures: **M. Marsala:** None. **S. Juhas:** None. **M. Hruska Plochan:** None. **D. Dolezalova:** None. **A. Miyanohara:** None. **S. Marsala:** None. **J. Juhasova:** None. **J. Motlik:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: NS 49177

Title: Gastrointestinal peptide dysregulation in acute spinal cord injury

Authors: M. C. P. GERAEDTS, M. S. MCLEAN, *G. M. HOLMES
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Abstract: Gastrointestinal (GI) dysfunction is a major physical and psychological burden for spinal cord injury (SCI) patients. We have shown that our model of experimental high-thoracic spinal cord injury (T3-SCI) mirrors the GI clinical presentation of neurotrauma patients, whereby T3-SCI shows diminished gastric emptying and dysmotility. The T3-SCI induced gastroparesis is due, in part, to an impaired vagally-mediated response to GI peptides. However, it is unknown whether nutrient-mediated peptide release from the GI tract is altered post-injury. We measured barrier integrity and GI peptide release from duodenal, ileal, and colonic explants of SCI and control rats at 1-day, 3-days, and 7-days post-surgery. Isolated intestinal explants were mounted in Ussing chambers, permitting measurement of transepithelial resistance (TER) and short-circuit current (ISC), and hormone secretion with or without apical glucose stimulation. 2 Hours after glucose stimulation, samples from the basolateral chamber where taken and stored at -80C until cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) were measured using ELISA assays. Prior to glucose stimulation, T3-SCI had no effect on TER measurements, but the ISC was decreased in T3-SCI animals at 3-days and 7-days post injury. This suggests that SCI does not change the integrity or passive transport of intestinal tissue, but it decreases active ion transport through the tissue. CCK secretion from duodenal tissue was increased beginning 1-day post-injury in T3-SCI animals compared to control animals. GLP-1 release from ileum was increased in T3-SCI animals 7-days post injury, whereas in colon tissue there was no differences between groups. PYY secretion from ileum was increased in T3-SCI animals 3 and 7 days post injury, and also here, there was no difference in PYY secretion from colon tissue between groups. Surprisingly, apical stimulation of ileal and colonic tissue with glucose resulted in a decrease in TER and rapid tissue death in T3-SCI animals. Also, glucose stimulation had no stimulatory effect on GI peptide release from intestinal tissue isolated from control animals, but it decreased peptide secretion from intestinal tissue isolated from T3-SCI animals. We conclude that T3-SCI affects GI peptide secretion in several areas of the intestine. The basal peptide

secretion is elevated before glucose challenge, but after glucose challenge, the peptide secretion decreases in injured animals. Surprisingly, the control operated animals do not respond to the glucose challenge, suggesting that the impact of vertebral laminectomy may also play a role in the secretion of GI peptides.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Metabolomic profile of cerebrospinal fluid and serum from acutely injured spinal cord patients

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Abstract: Background: Therapeutic development for spinal cord injury (SCI) is severely hindered by the difficulty in conducting clinical trials, which to date have relied solely on functional outcome measures for patient enrollment, stratification, and evaluation. Not only are these measures insensitive, but also are impossible to perform in many SCI patients. Biological biomarkers that accurately classify injury severity and predict neurologic outcome can offer a paradigm shift in the way SCI clinical trials are conducted. **Objectives:** The objectives of this study are to: 1) characterize the temporal metabolomic profile of cerebrospinal fluid (CSF) and blood after SCI, and 2) determine the relationship between CSF and serum profiles to identify serum biomarkers that reflect the changes within the CSF. **Methods:** Matched blood and CSF samples were used from a unique Canadian prospective multi-center trial in which CSF and blood from acute SCI patients are being collected and banked (Canadian Multicenter CSF Pressure and Biomarker Study, or "CAMPER"). Importantly, these patients are recruited prospectively and thus the biological data can be matched with well-documented clinical

outcomes. We performed an untargeted metabolomic analysis using high-performance isotope labeling liquid chromatography mass spectrometry (LC-MS). **Results:** Analysis of the LC-MS data revealed over 900 unique metabolites within the blood and CSF. For many metabolites, the relative intensity was higher for CSF samples collected at later time points after injury (48 compared to 24 hours). We are currently in the process of examining CSF and serum samples collected from SCI patients with different AIS grades. Chemometric and statistical analyses will determine which key metabolic pathways or networks are significantly altered in SCI, the extent of the correlation between network changes and SCI pathology, and identify potential biomarker candidates within the metabolite profiles. **Conclusion:** Metabolomic analysis of CSF and serum will further our understanding of the pathophysiology of SCI and potentially lead to the identification of reliable biomarkers for SCI. As trauma to the spinal cord is most directly reflected in the surrounding CSF, a comparison between blood and CSF is particularly valuable from a clinical perspective to interpret which changes within blood are due specifically to the SCI.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Serum and cerebrospinal fluid microrna biomarkers in a porcine model of spinal cord injury

Authors: *S. TIGCHELAAR[1], F. STREIJGER[1], C. NISLOW[2], S. SINHA[2], N. MANOUCHEHRI[1], K. SO[1], K. VAN KEUREN-JENSEN[3], I. MALENCIA[3], A. COURTRIGHT[3], T. BEECROFT[3], B. KWON[1,4]
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Abstract: Introduction: Currently, there are no effective treatments available for patients with spinal cord injury (SCI). There is an urgent need for both the scientific development and clinical validation of novel therapies for acute SCI. Biomarkers would potentially provide a more

objective, biological basis with which to stratify injury severity than our current functional outcome measures. Furthermore, establishing biological markers that can be evaluated after SCI, and subsequent therapeutic interventions in both animal models and human patients would provide important guidance for the translation of novel treatments. Objective: The objective of this study is to perform genome wide analysis of microRNAs in pig blood and CSF and determine their utility as biomarkers for SCI. Our interest in microRNAs stems from their specific spatial, temporal and cellular-level expression, as well as their stability within blood (making it possible for blood samples to be utilized in order to measure markers specific to the injured central nervous system). Method: Female Yorkshire pigs underwent a T10 spinal cord injury using a weight drop contusion impactor followed by compression for 5 minutes. CSF and serum samples were obtained daily over a period of 7 days. Extracellular miRNAs were isolated and sequenced using the Illumina MiSeq system and the generated data was aligned using miRDeep2 and tested for differential expression with DESeq software. Results: Using next generation sequencing, we were able to detect the expression of 719 miRNAs in pig samples of CSF or serum at 24-hours post injury. Amongst the most highly expressed miRNAs in pigs were miR-486, miR-26, miR-181, miR-10, and miR-92. MiR-486 has been shown to play a significant role in regulating SCI pathology. A validated target of miR-486 is NeuroD6, a protein important for neuronal differentiation and oxidative stress response. MiR-26 has been identified as an astrocytic miRNA; miR-181 is a regulator of pro-inflammatory mRNAs such as tumor necrosis factor and IL-1β and miR-92 has been identified as a diagnostic biomarker for severe brain injury. Conclusion: Initial sequencing data confirms the presence of miRNA implicated in CNS trauma in CSF and serum sample collected from paralyzed pigs. This preclinical study in our pig model of SCI has provided the rationale for moving forward with further studies using extremely valuable CSF and serum samples collected from human patients with SCI. By establishing these biomarkers in the pig and determining similarities with human SCI, we foresee generating a panel of markers that can be used both preclinically and clinically to evaluate treatment response.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Characterization of peripheral blood mononuclear cells in chronic spinal cord injury subjects

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Abstract: Introduction: Traumatic spinal cord injury (SCI) affects more than 12,000 Americans annually. Acutely after traumatic SCI, intraspinal and peripheral inflammation exacerbates the primary injury zone and promotes secondary tissue damage. Less is known about inflammation in the chronic phase of SCI, but long-lasting changes in local and systemic immune function have been proposed to impact functional outcomes and secondary complications of living with SCI. For example, we recently discovered that the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) and other inflammatory mediators are elevated in the circulation of chronic SCI subjects (Stein et al Archives of Physical Medicine and Rehabilitation, 2013). Here, we are examining the hypothesis that chronic SCI subjects have additional immune system changes, including alterations in the distribution or activation of peripheral blood mononuclear cells (PBMCs). To test this hypothesis, the objective of this study was to profile populations of circulating PBMC subsets in chronic SCI and age/gender matched uninjured subjects. Methods: This prospective, IRB-approved, observational pilot study of adult SCI subjects was performed in an academic medical center. "Chronic" SCI was defined as > than 1 year from initial injury. PBMCs were isolated from blood by Ficoll density gradient. Multicolor flow cytometric analysis of PBMCs was performed to examine the number and percentage of B cells, T cells, and Treg cells. Results: Uninjured (N=4) and SCI (N=6) subjects included 1 female in each group. Uninjured and SCI subjects were of similar ages (mean±sem, range): (55±5, 43-64 and 57±3, 45-64 years) ($P<0.61$). Mechanisms of injury for SCI subjects were: Fall (n=2), Sports (n=4). SCI subjects had an ASIA Impairment Scale (AIS) grade A. In this small group of subjects, our initial analysis did not reveal any difference in the percentage of CD3-CD19+ B cells, CD3-CD19+CD27- naïve B cells, CD3+CD4+ T cells, CD3+CD8+ T cells, or CD4+CD25+ Treg cells. Studies are ongoing to examine these and more specific PBMC populations in additional subjects. Future studies will also examine potential alterations in the activation state or function of PBMC subsets in chronic SCI as compared to uninjured control subjects.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Miami Project to Cure Paralysis

Buoniconti Fund

Title: In toto imaging of AAV-labeled axons after spinal cord injury using light sheet and confocal microscopy

Authors: *C. SODERBLOM, D.-H. LEE, P. TSOULFAS, J. K. LEE
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Abstract: Failure of axons to regenerate is the primary reason for paralysis after spinal cord injury (SCI). Thus, discovering mechanisms to promote axon regeneration has been an intense area of research. A technical challenge has been visualizing axon trajectory in the injured spinal cord that can provide clear origin-target information. Recent advances in tissue clearing methods have made it possible to overcome this hurdle, but all previous studies have been performed with transgenic mice in which the axons were prelabeled with green fluorescent protein (GFP). Thus, while these studies have provided a proof-of-concept, a more practical approach to investigating axon regeneration requires axon tracing. In this study, we labeled different axon tracts using adeno-associated viruses and performed tissue clearing to image the axons in toto using light sheet and confocal microscopy. Using mouse and rat models of SCI, our study demonstrates the promising potentials but also certain limitations in applying these methods to investigate axon regeneration.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Support: Advancing a Healthier Wisconsin

Title: Detecting acute neuronal injury with diffusion MRI: A simulation study

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Abstract: Acute injury to axons and dendrites results in a systematic sequence of degeneration. The formation of focal enlargements and constrictions (i.e. beading) is thought to represent the earliest stage of Wallerian degeneration and is a nearly ubiquitous morphological feature of acute neuronal injury. Although the therapeutic implications of beading are unclear, beading represents a potential diagnostic and prognostic indicator of injury to the central nervous system. Beading is observed *in vitro* and *in vivo* experimental preparations in animal models, but a noninvasive method would have important clinical diagnostic implications. One advanced magnetic resonance imaging technique, diffusion tensor imaging (DTI), has been shown to be a sensitive marker of axonal and dendritic injury in numerous models of trauma and disease. However, DTI is not a specific marker of axonal injury in many situations, since it can be confounded by other tissue properties, such as multiple intersecting fiber tracts (i.e. "crossing-fibers"), or other pathologies, such as edema, inflammation, gliosis, and others. A novel diffusion MRI method, the double pulsed field gradient (dPFG), has been shown to be insensitive to the effects of crossing fibers and has the potential to specifically detect beading of axons and dendrites non-invasively with greater specificity than DTI. In this work, we simulated the DTI and dPFG experiments in geometrical models of normal and beaded axons in a variety of different fiber configurations. Whereas fractional anisotropy (FA), the most commonly used measure derived from DTI, is reduced in beaded axons, it is also reduced with edema and in crossing fibers. Thus, FA changes alone are ambiguous with respect to the underlying biology. On the other hand, a metric derived from the dPFG, eccentricity, is reduced as a consequence of beading, but is largely immune from edema and crossing fibers. Collectively, the results of the computational simulations demonstrate that the dPFG is a unique and specific marker of acute neuronal injury, which is likely to have important implications for clinical diagnosis in a variety of acute insults to the brain or spinal cord.

Disclosures: M.D. Budde: None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Support: NIH RO1 NS060784

SHC 84050

SHC 85200

Title: Combined neurotrophin treatment promotes regeneration of multiple sensory modalities after dorsal rhizotomy

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Abstract: Gene therapy offers a promising approach to provide neurotrophins at the injury site and thus enhance axonal regeneration after a nervous system injury. For investigating regeneration, we crush the L4/L5 dorsal roots and inject lentivirus into the spinal cord at the dorsal root entry zone (DREZ) of L4/L5 nerves immediately after injury. We have previously shown lentiviral expression of NGF and artemin at the dorsal root entry zone produces robust regeneration of calcitonin gene related peptide positive (CGRP+) axons. Artemin, but not NGF, produced a modest but significant regeneration of nonpeptidergic isolectin B4 positive (IB4+) axons. Surprisingly, artemin did not support regeneration of myelinated sensory afferents, as previously reported. In this study, we expressed GDNF or GDNF/Artemin at the DREZ using the lentiviral expression system. GDNF did not elicit regeneration of CGRP+ axons, but produced a significant regeneration of IB4+ axons and enhanced the regeneration of cholera toxin-B labeled myelinated sensory afferents across the DREZ. Combinatorial expression of GDNF and artemin showed further enhanced regeneration of IB4+ and CTB labeled myelinated axons, demonstrating a positive cooperativity between these factors. Thus, using a combination of lentiGDNF and lentiartemin, we were able to reconstruct multiple sensory axonal modalities anatomically. Physiologically, as the IB4+ axons respond to the high threshold input, only 50% of the GDNF group showed a return of thermal nociception. In contrast, the anatomical

regeneration of CTB labeled axons did not support any functional restoration, and appeared to remain within the dorsal horn. We evaluated the return of paw pressure sensation and proprioception using Ugo-Basil Analgesymeter and grid walk test and found no improvement. In a separate experiment, we were able to enhance regeneration of myelinated sensory afferents into lamina VIII by overexpressing Rheb within L4/L5 DRG neurons and concomitantly expressing neurotrophin NT3 at the DREZ. Rheb alone in the DRG did not induce any regeneration while expression of NT3 alone in the DREZ produced a superficial regeneration of these axons. Altogether, our data reinforces the importance of combinatorial approaches in enhancing CNS axonal regeneration.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Support: NIH NRSA F31NS077750

Title: The effect of spinal cord injury on bladder-specific nodose ganglion neurons

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Abstract: We have previously demonstrated in male rats anatomical evidence that the vagus nerve provides afferent innervation to the urinary bladder and that the extent of the vagal supply was similar to parasympathetic innervation from spinal sources (L6 DRG). The equivalent proportion of innervation to the bladder from both vagal and spinal sources may signify separate functional roles for these afferent fibers. To better understand the functional significance of bladder-specific neurons in the nodose ganglion (NG, sensory cell bodies of the vagus nerve), we examined their immunohistochemical profile in the naïve and chronically injured state using a complete transection model. We also examined spinal phenotypic differences in the L6 DRG as a comparison with the NG. In male Wistar rats, a subset of animals received a complete spinal cord transection at the level of T8. After a period of 5 weeks, the retrograde tracer, Fast DiI, was injected into the lining of the bladder and given 10 days for transport to the NG and DRG. The

NG and L6 DRG were processed for multi-label immunohistochemical analysis for the expression of P2X3 receptors and IB4 binding. Initial findings of the NG demonstrated, in the transected animals, a significant increase in the number of neurons expressing P2X3 and a significant decrease in the number of neurons binding IB4 compared to naïve controls. These results suggest both an inflammatory and stress response respectively may be occurring in NG neurons. In these two distinct NG subsets, we found that more than half of the neurons received bladder input in each group. Additionally, the NG demonstrated significantly greater bladder-specific/IB4+ neurons compared to the L6 DRG suggesting two distinct neurochemical profiles exist for visceral afferents between vagal and spinal sources. The high prevalence of bladder-specific neurons in the P2X3 and IB4 subsets demonstrates potential bladder transduction mechanisms through the vagus and its likely role in visceral nociceptive processing.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Support: National Rehabilitation Center Grant

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Title: Effects of repetitive transcranial magnetic stimulation on the functional recovery of patients with central cord syndrome

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Abstract: The aim of this study was to delineate the effect of repetitive transcranial magnetic stimulation (rTMS) on the functional recovery of upper extremity in patients with central cord syndrome (CCS). Fourteen CCS patients were treated high frequency (20Hz) rTMS over the motor cortex for 5 days. All patients also received conventional occupational therapy during

rTMS treated period. Clinical evaluations including neurological assessment using International Standard for Neurological Classification of Spinal Cord Injury (ISNCSCI), hand functional assessments using the Jebsen hand function test and O'conner finger dexterity test were performed before rTMS initially and followed up 1 month after rTMS treatment. Diffusion tensor imaging and tractography of cervical spinal cord were also performed, and fractional anisotrophy (FA), apparent diffusion coefficient (ADC), imaginary crossing fiber numbers and the connection rate of fiber tracts were obtained in each patient. The motor score of upper extremity was improved during follow-up period of all treated side (100%) and more than half of non-treated side (75%) of CCS patients. The writing score of Jebsen hand function test were significantly increased more in treated side compared with non-treated side 1 month after rTMS treatment. The initial FA values of injured cervical spinal cord and the connection rate crossing injured cervical spinal cord in cervical spinal cord were correlated with the clinical motor score of upper extremities. There were no adverse effects during rTMS therapy and follow-up period. We tried to perform high frequency rTMS to CCS patients for the first time, and rTMS might enhance functional restoration, especially fine motor task performance of the upper extremity in CCS patients without any adverse effect.

Disclosures: **J. Hyun:** None. **T. Kim:** None. **S. Kim:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Support: NIH Grant PO1 NS055976

Title: An animal model for spinal cord concussion and repeated injury

Authors: ***Y. JIN**, J. BOUYER, C. HAAS, I. FISCHER
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Abstract: Spinal cord concussions, which occur during vehicular accidents, falls, and sport activity, are characterized by a transient loss of motor and sensory function that usually resolves without permanent deficits. Unlike brain concussions, they have received little attention despite the potential for repeated injury leading to permanent neurological sequelae. Importantly, there are no models of spinal concussion to study the anatomical and functional sequelae of single or

repeated injury, and consequently, there is poor understanding of the short- and long-term consequences of such injuries. We have first examined the established mild thoracic contusion model utilizing the NYU impactor, but found that the severity of the injury was not compatible with the clinical presentation of spinal concussion. We subsequently developed a new model that shows transient deficits with minimal anatomical damage and used the model to study concussion and repeated injury. Rats received a very mild (50kdyn, IH impactor) contusion at C5 and were separated into two groups three weeks after the initial injury - C1, which received a second, sham surgery, and C2, which received a second injury at the same site. Animals received weekly behavioral tests - BBB, CatWalk, cylinder, and Von Frey. Analysis of locomotor activity by BBB demonstrated rapid recovery to near-normal function by 1wk after the first and second injury, which was confirmed using the more detailed CatWalk analysis. The cylinder test showed that single contusion did not induce significant deficits of the affected limb, but that repeated injury resulted in significant alteration in paw preference, with animals favoring the unaffected limb. Intriguingly, Von Frey analysis demonstrated an increased sensory sensitivity in the contralateral hindlimb in the C2 group vs. the C1 group. Anatomical analyses revealed that while the lesion volume of both groups was minimal, the area of spared white matter in the C2 group was significantly reduced 1-2 mm rostral to the lesion epicenter. Reactive astrocytes were present in both groups, with the majority found around the lesion epicenter in the C1 group, whereas the C2 group demonstrated increased reactive astrocytes extending 1mm caudal to the lesion epicenter. Macrophages accumulated within the injured, ipsilateral spinal cord, with significant increases 2-3mm rostral to the epicenter in the C2 group. Our model is designed to represent the clinical presentation of spinal cord concussion and highlight the susceptibility and functional sequelae of repeated injury. Future experiment will examine the temporal and spatial windows of vulnerability for repeated injuries.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Lewis, Fischer 344 and Sprague-Dawley rats display differences in lipid peroxidation, motor recovery and neuronal survival after spinal cord injury

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Abstract: Lewis (LW), Fischer 344 (F344) and Sprague-Dawley (SPD) are the rat strains that are most commonly used for spinal cord injury (SCI) research. However, these strains have subtle differences in physiological and immune responses. These variations could alter the morphological and functional recovery after SCI among strains. For this purpose we decided to evaluate one of the most detrimental events after SCI, lipid peroxidation (LP) and its effect on rubrospinal neuron (RSN) survival and functional locomotor recovery. Our study consisted of three experiments which all had three groups (LW, F344, and SPD; 78 animals): (1) the three strains (n = 8/group) were subjected to laminectomy of the T9 vertebrae (sham surgery) and 72 hrs after by-products of LP were quantified; (2) strains (n = 8/group) sustained a moderate spinal cord contusion at T9 (MASCIS impactor) and after 72 hrs, LP was quantified; (3) the three strains (n = 10/group) were subjected to SCI and were evaluated weekly using the BBB locomotor scale for 60 days. At the end of the follow-up period, subjects were randomly selected from each group (n = 4/group) for RSN survival analysis. Although LP-products were barely detected in sham-operated rats, F344 and SPD presented lower LP levels as compared to LW ones. The levels of LP after SCI in LW rats were significantly higher [406.81 ± 43.5 fluorescence units/g of tissue; mean ± standard deviation (SD)] in comparison with either F344 (233.69 ± 41.98) or SPD rats (220.40 ± 14.20; $P < 0.01$, one-way ANOVA followed by Tukey's test). The BBB score of LW rats was also significantly lower (3.2 ± 0.3; mean ± SD) than the one demonstrated by F344 (4.9 ± 0.8) or SPD animals (5.1 ± 0.7; $P < 0.001$, repeated measure one-way ANOVA followed by Bonferroni's test). RSNs were barely detected in LW rats; they showed a mean of 12 ± 5 surviving cells (% from sham-operated rats: 5.1 ± 2; mean ± SD), while F344 and SPD showed 30 ± 7 (%: 10.4 ± 2) and 38 ± 5 (%: 12.6 ± 4) respectively. The present findings are of relevant interest to SCI research since strain differences must be considered for future investigations, especially those evaluating LP or motor recovery after SCI.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Title: The role of mTOR and STAT3 in the intrinsic growth of rubrospinal neurons

Authors: *K. M. KEEFE, Y. LIU, G. SMITH
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Abstract: It is well known that regeneration in the mature central nervous system (CNS) is rare. In order to repair damage with sufficient vigor to re-establish functional connections, the neuronal cell body must be able to turn on genes needed to form new growth cones, produce proteins and growth factors, and elongate and repair damaged axons. Adult CNS neurons are particularly resistant to this type of activation, but there is evidence to show that these 'growth programs' can be stimulated in the right circumstances. In this study, we explore upregulation of growth programs in rubrospinal neurons via stimulation of the mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) pathways. It is thought that directly priming neurons with a transcriptional activator such as STAT3 combined with induction of protein translation mediated though mTOR activation will produce a robustly active growth state. This is accomplished via injection of adeno-associated viruses (AAVs) encoding ras homolog enriched in brain (AAV-Rheb), a direct positive regulator of mTOR, and AAV-STAT3, unilaterally into the red nucleus of a female rat, followed by a unilateral hemisection of the thoracic spinal cord level 8 contralateral to the injection site. At 5 weeks post-injury, we found that animals injected with AAV-Rheb/AAV-STAT3 show greater incidence of regrowth such as sprouting, abnormal axon morphology, and growth into and beyond the lesion site when compared to controls injected with AAV-GFP. This study highlights the importance of mTOR and STAT3 in axonal growth of the rubrospinal tract after injury.

Disclosures: K.M. Keefe: None. **Y. Liu:** None. **G. Smith:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Program#/Poster#: 422.16/Y11

Topic: C.10. Trauma

Title: Effect of the ketogenic diet on expression of anti-inflammatory genes in a rodent model of SCI

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Abstract: Introduction: Recently, we demonstrated in an animal model of SCI that a low carbohydrate, high fat ketogenic diet (KD), starting 4 hours following injury improves both gross and fine forelimb function and enhanced neuroprotection. Importantly, after returning to a standard diet after 12 weeks of KD treatment, the improved forelimb function remained stable. Although this study emphasizes an important effect of food on functional recovery after SCI, further work is necessary to determine the mechanisms of action. As the underlying mechanisms KD become better understood, it will be possible to develop alternative strategies that produce similar or even improved therapeutic effects and could potentially have significant impact on improving thewellness of individuals living with SCI. **Objectives**: The objective of this study is to determine the temporal anti-inflammatory effects of KD using a rat model of SCI. **Methods**: To test study this, a cervical hemicontusion injury model in rats was used. Starting 4 hours after SCI, animals were fed either a regular carbohydrate-based rodent diet, or 2) a KD diet with ratio of fat to carbohydrate and protein of 3:1. To examine the effect of KD on inflammation, we used microarray methods to examine gene expression 2 days, 7 days, and 6 weeks after SCI. **Results**: Microarray analysis showed that many inflammation-related genes were robustly changed in the cervical spinal cord of KD treated animals at 2 days, 7 days and 6 weeks after injury. Notably, the expression changes of genes involved in inflammation were largest at 6 week post-injury. Among the 152 genes, 23.0% (35/152) of them encode protein associated with inflammatory response and 15.1%(23/152) with immune cell trafficking. Immunohistochemistry and western blotting techniques are currently being performed to confirm the microarray data. **Conclusion**: Our data provide some insights into the complex cascade of cellular changes in the spinal cord induced by KD, some of which may contribute to its protective effect after SCI. KD treatment of injured rats result in prominent changes in genes encoding proteins involved in inflammatory signal pathways. Hence regulation of inflammatory response could be one of the principal mechanisms of KD-induced neuroprotection following SCI.

Disclosures: X. Wu: None. **O. Jang:** None. **J. Zhu:** None. **J. Liu:** None. **W. Tetzlaff:** None. **F. Streijger:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Bilateral contusion-compression model of incomplete traumatic cervical spinal cord injury

Authors: *N. FORGIONE[1], S. K. KARADIMAS[1,3], W. FOLTZ[4,2], K. SATKUNENDRARAJAH[1], A. LIP[3], M. G. FEHLINGS[1,3]
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Abstract: Approximately two thirds of traumatic spinal cord injuries (SCIs) result in incomplete lesions; the majority of these injuries occur in the cervical spinal cord causing devastating neurological impairments. Despite a defined need, we lack a clinically relevant injury model that replicates the pathomechanisms of this injury, and that can be used to test potential therapies. To address this key knowledge gap, we characterized a contusion-compression model in rats that generates a bilateral, incomplete injury. We described the effects of moderate (18 gram) clip-compression injury at cervical level C6 over an 8-week recovery period. First, we quantitatively assessed the effects of injury on gray and white matter sparing, lesional tissue, and cavity formation at 2, 4, 6 and 8 weeks post-injury. Next, magnetization transfer (MT) and T2-weighted magnetic resonance imaging (MRI) were used to analyze lesion dynamics *in vivo*. Our next goal was to assess neurobehavioral recovery in C6 injured rats. We used BBB, Catwalk, and grip strength to test forelimb and hindlimb motor function. Overall, these analyses showed that while motor function was significantly reduced in injured animals, there was sparing and recovery. Sensory evoked potentials (SEPs) recorded from the forelimb demonstrated decreased amplitude and increased latency at 8 weeks post-injury. Hoffman (H) -reflex recorded from the hindlimb was indicative of increased spasticity, thus recapitulating below-level spasticity seen in clinical SCI. The sparing of motor function accompanied by improvements over time mimic the symptoms seen in humans with incomplete cSCI. Therefore we have developed a clinically relevant model of cSCI that will contribute to improved success in the translation of putative therapies.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Identification of genes related to ketogenic diet-induced beneficial functional and neuroprotective effects following SCI

Authors: *W. TETZLAFF[1,2], J. ZHU[2], S. CEN[2], J. LIU[2], A. HAEGERT[3], S. LE BIHAN[3], F. STREIJGER[2]
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Abstract: Introduction: The current dietary recommendations for the management of spinal cord injury (SCI) focus mostly on food that has high carbohydrate and low fat content. However, data from our own lab suggest that such carbohydrate-based diets may actual dampen neurological outcome following SCI. Contrary to our current clinical practice, we reported that feeding a low carbohydrate, high fat diet, a so-called ketogenic diet (KD), improved reaching ability that involves fine manipulation of the distal muscles of the digits and supination. Furthermore, we initiated KD not until 4 hours after injury and this time window of intervention differs from the majority of drug treatments for SCI, which have been studied with only little to no delay, which is not realistic in the overwhelming majority of acute SCI patients. **Objective:** As of today, the mechanisms of the beneficial effects of KD following in SCI are unknown. Therefore, the goal of this study is to identify differentially expressed genes that may underlie the KD-induced functional and neuroprotective effects using micro-array techniques. **Methods:** To identify the effects of KD on the overall pattern of mRNA expression after acute SCI, we collected tissue from rats injured with the Infinite Horizon spinal cord impactor at the C5 level. SCI rats were treated with either SD or KD for 48 hours, 7 days or 6 weeks. 200 ng of total RNA was used and labeled using the Low Input Quick Amp Labeling Kit. Gene expression profiles were analyzed using direct hybridization assay (Illumina). Ingenuity Pathway Analysis software was used to identify molecular pathways and networks. **Results:** KD affects the gene expression of many astrocyte and endothelium-derived vasoactive mediators such as Endothelin Converting Enzyme (ECE), the highly sensitive, vasodilatory PGE2 receptor and cytochrome P450 monooxygenases (CYP2b19). Furthermore, we observed downregulation of phospholipase A2 (PLA2G4E) and prostaglandin receptors (PTGER1 & PTGFR) in the spinal cord of KD treated

SCI-rats, genes that have a diversity of biological functions. These results prompted us to analyze the effects of these genes in KD-induced beneficial effects. Selected RT-PCR and western blotting confirmations are currently being performed and will be presented. **Conclusion:** Overall, the results of our study provide an initial picture of the molecular mechanisms. As the underlying mechanisms of dietary approaches such as the ketogenic diet become better understood, it will be possible to develop alternative strategies that produce similar or even improved therapeutic effects and could potentially have significant impact on neurological outcome following acute SCI.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: The relevance of hyaluronidase-4 and astrocytes in a rat spinal cord hemisection model

Authors: *Y. SHIMIZU[1], T. OKUDA[1], N. KAWAHARA[1], N. KATO[2], Y. ISHIGAKI[3], T. MATSUMOTO[1]
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Abstract: [Purpose] Chondroitin sulfate proteoglycans (CSPGs) are known as an axon reproduction inhibitor that is derived from the reactive astrocyte in the glial scar. Our previous study revealed that these CSPGs remain constant in the injured part in the rat spinal cord contusion model. In the hemisection model, by constrast, CSPGs expression reaches its peak 3 weeks after the hemisection and decreases thereafter. The Chondroitinase ABC (ChABC) of bacterial origin is known as exogenous digestive enzymes of CSPGs. CSPGs decrease when the ChABC is administered to the injured part of the spinal cord contusion model. The decline of CSPGs expression in a hemisection model may be attributed to a gradual expression of an endogenous enzyme with the chondroitinase activity. Hyaluronidase-4 (Hyal-4) is reported as a mammalian enzyme which specifically breaks down chondroitin sulfate (CS) containing GAG chains. Our data so far demonstrated a co-localization of reactive astrocytes and Hyal-4 in the region surrounding the site of CSPGs expression in the hemisection model, suggesting that

reactive astrocytes produce Hyal-4. Thus, this presentation aims at examining how Hyal-4-positive regions overlap with expression of CSPGs and the astrocyte marker GFAP. [Methods] Experiments were conducted in female Sprague-Dawley rats (10-week-old) with spinal cord hemisection. Frozen sections of the injured spinal cord were prepared after perfusion with 4% paraformaldehyde on day 4 or at 1, 2, 3, 4, 5 or 6 weeks post hemisection. Immunofluorescence studies were performed using the following primary antibodies: CS56 for CSPGs, anti-Hyal-4 antibody for Hyal-4 and anti GFAP antibody for astrocytes. The secondary antibodies were conjugated with Alexa 488 or Alexa 594. Images were acquired using BZ-9000(KEYENCE). [Results] The majority of the GFAP-positive area overlapped with the Hyal-4-positive region, and GFAP expression was located to a much lesser extent in the CS56-stained area near the injured site. Furthermore, some of the GFAP-expressing cells were Hyal-4-positive.[Conclusions] The data agreed with the previous finding that reactive astrocytes produce CSPGs. We also suggested that reactive astrocytes express Hyal-4. We propose that reactive astrocytes may fine-true the micro-enviroment around the injured site by regulating production of both CSPGs and its endogenous digesting enzyme Hyal-4 as well.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Craig H. Neilsen Grant 260637

Thomas Jefferson University, Jefferson School of Health Professions, Department of Physical Therapy

Title: Inter-regional intrinsic brain activity effects in pediatric spinal cord injury

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Abstract: Purpose: To evaluate cerebral inter-regional functional reorganization in children with different levels of spinal cord injury (SCI) using intrinsic activity connectivity measures. **Methods:** We measured changes in cerebral inter-regional correlation in six female and 12 male participants with SCI, average age 16.3 years, during two fMRI sessions gathered 2-48 hours apart. During each session participants were instructed to keep their eyes open while maintaining fixation. The resulting time series were processed to mitigate effects of head motion and other sources of physiological noise. After spatial normalization functional connectivity of selected sensorimotor regions were quantified using seed-voxel analysis in complete (AIS A) and incomplete (AIS B, C, and D) SCI participants. **Results:** We observed effects on inter-regional connectivity related to the level of SCI (paraplegia versus tetraplegia), with primary motor cortex on both sides showing increased coupling with extrastriate visual cortex with damage at cervical compared to thoracic levels. **Conclusion:** Inter-regional functional connectivity assessment is a practical and sensitive technique to characterize the cerebral effects of SCI in children. Inter-regional correlations of intrinsic cerebral activity reveal increased coupling in visual and motor cortical regions related to the level of damage.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: C.10. Trauma

Title: Spinal cord injury-induced plasticity in sensory nociceptive processing and autonomic function

Authors: K. K. MARTIN, D. J. NOBLE, S. HOCHMAN, *S. M. GARRAWAY
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Abstract: Spinal cord injury (SCI) results in several devastating effects which typically include the impairment of sensory and autonomic function. For instance, both the development of pain and compromised respiratory function are critical consequences of SCI. Despite the prevalence of these maladaptive conditions, the underlying mechanisms are poorly understood. Also, although previous reports have suggested that there are parallels in impaired sensory and autonomic signaling, a thorough investigation into their interactions after SCI has not been

shown. To examine sensory-autonomic dysfunction after SCI, we used adult Long Evans rats with a T8 moderate contusion injury (IH Impactor, 150 kdyne) or sham surgery to compare SCI-induced plasticity in respiratory rate and induced pain responses. Beginning 24 hours after injury, we recorded respiratory rate and movement related behaviors using highly sensitive non-contact electric field sensors (EPIC, Plessey Semiconductors). Recordings were obtained before, during and after behavioral pain tests which included the measurement of hind-paw mechanical allodynia (withdrawal response to von Frey filaments), brush-induced at-level mechanical allodynia and nociceptive tail-flick latency. Because changes in respiratory rate represent a physiological autonomic response to pain, results of this study will provide an important integrative dimension to pain plasticity not typically studied after SCI. Preliminary results show that SCI rats have a higher basal respiratory rate that is maintained for several weeks after injury. Moreover, unlike sham rats, SCI rats increased their respiratory rates in response to mechanical stimulation, consistent with an observed mechanical allodynia. Studies are underway to further relate SCI-induced allodynia to changes in autonomic function, including heart rate and cardiorespiratory synchronization.

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Poster

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Topic: C.10. Trauma

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Title: The effect of intrapspinal microstimulation parameters on movement thresholds in normal and spinal cord-injured rats

Authors: *S. B. FROST[1], C. L. DUNHAM[2], S. BARBAY[2], D. W. MCNEAL[2], D. KRIZSAN-AGBAS[1], M. K. WINTER[3], D. J. GUGGENMOS[2], R. J. NUDO[2]
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Abstract: This study was conducted to determine the effect of various intraspinal microstimulation (ISMS) parameters on the minimum current required to evoke a visible joint movement (i.e., movement thresholds) in normal and spinal cord-injured (SCI) rats. Experiments were carried out in four adult, male, Fischer 344 rats using commercial laboratory equipment (*Tucker Davis Technologies*). Subjects consisted of two normal (uninjured) rats and two rats that had received a 250 kDyn contusion injury to the thoracic spinal cord at level T8-T9. SCI rats demonstrated a severe deficit after injury (BBB avg 10) and were recovered at least four weeks prior to the ISMS procedure. During this procedure, a silicon-based microelectrode array (*NeuroNexus Technologies*) with stimulus site impedance in the range of 120–140k at 1kHz was inserted into the spinal cord at the lumbar level. Each stimulus site position ranged from ~500–2,800μm below the surface of the posterior spinal cord, with the medio-lateral position ~250–1,500μm lateral to the midline. All electrophysiological experiments were conducted under ketamine anesthesia. Both monophasic (with passive discharge) and biphasic current pulses were used to determine the relative efficacy of each ISMS mode for evoking hindlimb movements. In each rat, movement thresholds were determined in at least 60 sites. The ISMS responses typically consisted of movements of the hip, knee, ankle or toes, with movement thresholds typically ranging from ~14–25μA. While monophasic anodic pulses resulted in slightly lower movement thresholds compared to monophasic cathodic pulses, there was no significant difference between these conditions. There was a trend toward lower thresholds with higher frequencies using anodic pulses, but these differences were also not significant. In a normal rat, movement thresholds using biphasic cathodic leading stimulation were significantly lower when using a 13 or 5 pulse train compared to single pulse train. However, even when using a single pulse, the movement thresholds averaged only 16 +/- 3.2μA. Average hindlimb biphasic stimulation movement thresholds were 15.7 +/- 1.1 μA and 18.7 +/- 0.9 μA in a normal and SCI rat, respectively. While movement thresholds in the SCI rat were statistically significantly higher, the actual difference was quite small (< 3μA). These results demonstrate that ISMS current thresholds for evoking hindlimb movement remain low in a rat model of spinal cord injury.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: D.10. Spinal Cord Injury and Plasticity

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Title: Delivery of autologous neurotrophin-producing fibroblasts promotes locomotion in the chronic spinal cat

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Abstract: Adult cats show limited locomotor capabilities following spinal transection. With body-weight support training, the animals recover stepping ability with weight-bearing plantar foot placement. We previously found that delivery of neurotrophins via xenografts of genetically modified rat fibroblasts promotes a similar motor recovery without training. Similar results were obtained in rodents using direct injection of viruses into the cord. Viral delivery raises clinical concerns regarding recombinant genetics, and xeno/allografts require immunosuppression that increases cancer risks. Furthermore, none of these studies has investigated the effects of delayed onset of neurotrophin delivery on recovery. Our study utilized autologous fibroblasts modified to express the neurotrophins BDNF and NT-3 grafted into the spinal cord following a complete transection at T11-T12. Fibroblasts were grafted at the time of injury, 2 weeks after injury, or 6 weeks after injury. Recovery of bipedal stepping on a treadmill was evaluated before and after injury and grafting. Kinematic evaluation indicated that grafting promoted recovery of treadmill stepping in the experimental groups. While control cats could only perform limited stepping at low (<=0.4m/s) speeds, grafted cats recovered stepping at all speeds up to 0.8m/s. Recovery was seen in many grafted cats as early as 3 weeks after injury, and all but one grafted cat were capable of stepping by 5 weeks after grafting. This recovery remained 12 weeks after grafting. Histological evaluation showed no regeneration through the lesion. We conclude that neurotrophin producing autografts are effective at promoting stepping even when delivered in a chronic spinal cord injury model.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Title: Quantitative proteomic analysis of cerebrospinal fluid after acute human spinal cord injury

Authors: *N. MANOUCHEHRI[1], J. C. ROGALSKI[2], F. STREIJGER[3], S. PERRY[2], C. BORCHERS[7], J.-M. MAC-THIONG[8], S. PARENT[8], S. D. CHRISTIE[9], C. S. BAILEY[10], R. F. BALSHAW[11,4], L. J. FOSTER[2,5], B. K. KWON[3,6]
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Abstract: The development and subsequent translation of effective therapies for spinal cord injury (SCI) is hampered by the paucity of information about the biology of injury in human patients, and the lack of biomarkers that could facilitate clinical trials. Such biomarkers of SCI would be helpful for precisely classifying the injury severity, predicting neurologic recovery, and providing surrogate outcome measures for evaluating the biological effects of novel therapies. Previously, we utilized ELISA techniques to identify 6 biomarkers of injury severity in CSF from acute SCI patients. To expand the breadth of this analysis and potentially identify additional biomarkers, we conducted a targeted proteomics analysis of human CSF samples utilizing advanced mass spectrometry techniques. Acute ASIA A, B, and C SCI patients were enrolled in a prospective clinical trial in which CSF samples were obtained through indwelling intrathecal catheters at 24, 48 and 72 hours post-injury. The injury levels ranged from C4 to T11. Further, CSF from 6 non-SCI patients undergoing lumbar laminectomy/discectomies was also tested. A targeted proteomics approach was employed using multiple reaction monitoring (MRM) mass spectrometry to measure the levels of more than 100 proteins across all patients. The specific proteins evaluated were selected based on previous analyses of human CSF from patients with other neurologic conditions such as traumatic brain injury, stroke, Alzheimer's, and Parkinson's disease. Method development was performed on Agilent 6460 and 6490 triple quadruple mass spectrometers, using synthetic proteotypic peptides corresponding to the previously discovered proteins of interest. The MRM assays developed provided the highest sensitivity transitions for a given peptide, the highest selectivity for a given protein, short analysis times and minimal sample volumes. From 283 optimized peptide candidates, 75 were found to be present in human post-SCI CSF test samples. These were combined into a single analysis method, allowing the quantitation of these low level proteins in human CSF in one experiment. In this study, we utilized an immune-depletion strategy in which 14 major high-abundance proteins are removed by commercially available multi-affinity columns, which enable

effective detection of unique low abundance proteins. We are currently in the process of examining the human CSF and serum samples at various time points after SCI. The insights from this study will help scientific researchers develop clinically applicable therapies for human patients, and provide clinical researchers with tools that will facilitate the testing of novel therapies.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: D.10. Spinal Cord Injury and Plasticity

Title: Chronic monitoring of intraparenchymal pressure and spinal cord blood flow following a contusive thoracic SCI in a porcine model

Authors: ***K. SO**[1], F. STREIJGER[1], N. MANOUCHEHRI[1], J. H. T. LEE[1], J. SOICHER[1,2,3], P. A. CRIPTON[1,2,3], B. K. KWON[1,4]
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Abstract: Introduction: To date, expeditious surgical removal of impinging bone fragments after traumatic spinal cord injury (SCI) is one of the few treatment options available for the management of acute SCI. Intuitively, removing extradural compression reduces pressure and restores cord perfusion. But while surgical decompression may provide relief of extradural pressure, the cord itself typically continues to swell, indicating a potential increase in intraparenchymal pressure. Indeed, we have recently demonstrated in a porcine model of SCI that intraparenchymal pressure continues to increase even after spinal cord decompression. The biological basis and physiologic consequences of such swelling are poorly understood. Therefore, the goal of the present study is to determine how intraparenchymal pressure changes in the cord as swelling occurs, and the resultant effect on spinal cord blood perfusion. **Method:** Miniature Yucatan pigs received a contusive SCI at T10/11, using a controlled weight drop device followed by 1-hr of sustained compression. Fibre-optic pressure sensors and laser

Doppler flowmetry probes were implanted caudally into the spinal cord at 2 and 4 cm from the injury site. The cord pressure (CP) and blood flow (BF) were continuously monitored for up to 7-days. **Results:** After a contusion/compression injury, the CP and BF adjacent to the injury site (2 cm) instantaneously increased and decreased, respectively. Decompression caused the CP to return to baseline and the BF to transiently increase above baseline, a hyper-perfusion effect. The CP then increased rapidly over the next 4 hours while the BF, returned back to baseline levels. Over the 7-day sampling period, the CP remained at an elevated state while the BF fluctuated around baseline levels. Further from the injury site (4 cm), the CP and BF changes were far less pronounced during the injury, compression and decompression periods. However, 3 hours after decompression, the CP began to rise while the associated BF decreased below baseline. Unlike the CP at the 2 cm site, the 4 cm site CP, while it remained elevated above baseline over the 7-day sampling period, it exhibited greater fluctuations. The BF at the 4 cm site also fluctuated more than the BF closer to the injury site and its direction of changed tended to oppose that of its associated CP. **Conclusion:** Our data demonstrates that following SCI, the intraparenchymal pressure of the spinal cord re-accumulates within hours of decompression. Additionally, our data suggests that the pressure changes in the spinal cord may have a deleterious effect on cord blood perfusion, but further investigation is warranted to better elucidate this dynamic relationship.

Disclosures: K. So: None. **F. Streijger:** None. **N. Manouchehri:** None. **J.H.T. Lee:** None. **J. Soicher:** None. **P.A. Cripton:** None. **B.K. Kwon:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 422.26/Y21

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NINDS 1R01NS076976a

Title: Olfactory ensheathing cells and fibroblasts differ their modification of the lesion site after complete spinal cord transection

Authors: R. R. KHANKAN[1], K. G. GRIFFIS[2], D. N. PEREZ[2], J. R. HAGGERTY-SKEANS[2], H. ZHONG[2], R. R. ROY[2], V. R. EDGERTON[2], *P. E. PHELPS[2]
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Abstract: To better understand the growth-promoting potential of olfactory ensheathing cells (OECs), we transplanted transgenic eGFP-expressing OECs or skin fibroblasts (FBs) 1 mm above and below a complete T8 spinal cord transection. This short-term study compared how OECs and FBs modify the injury site at 1, 2, and 4-weeks post-lesion and analyzed 5-6 rats per cell type at each time-point. The size of the lesion core was smaller in OEC than in FB-treated rats at 1-2 weeks, but lesion sizes did not differ by 4-weeks post-injury. OECs migrated from the injection site into the lesion core as streams of cells organized into networks that intermingled with astrocytes, whereas FBs formed only a solid sheet of cells that did not integrate into the GFAP-positive glial scar. Despite the limited number of surviving transplanted cells, we evaluated the extent of neuronal survival and axon sparing near the GFAP scar border. Neurons were located closer to the scar border and more serotonergic axon bundles crossed the scar border in OEC than in FB-treated spinal cords at 1-2 weeks post-transection. We next asked if microglia and macrophage expression of ionized calcium binding adapter molecule-1 (Iba1) differed in its distribution and interactions with OEC and FB transplants. The intensity of Iba1-immunoreactivity in the lesion core of OEC and FB-treated groups was similar. Iba1 reactivity, however, was lower in OEC than FB-treated spinal cords at the glial scar border and within the rostral and caudal stumps. These data imply that OECs modulate the immune response after a spinal cord injury and that these immunological interactions enhance neuronal survival and axon sparing.

Disclosures: R.R. Khankan: None. **K.G. Griffis:** None. **D.N. Perez:** None. **J.R. Haggerty-Skeans:** None. **H. Zhong:** None. **R.R. Roy:** None. **V.R. Edgerton:** None. **P.E. Phelps:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Taiwan National Science Council grants 101-2320-B-006-008

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Title: The roles of CCAAT/enhancer binding protein delta (CEBPD) in astrogliosis after spinal cord injury

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Abstract: Spinal cord injury (SCI) is a devastating disease that results in disruption of microstructure in the cord and is followed by limited neuronal regeneration and functional recovery. After SCI, astrocytes, the most abundant glial cells in the central nervous system, become reactive and hypertrophy. Increased number of reactive astrocytes leads to astrogliosis which can be observed in a variety of chronic neurological and neuroinflammatory diseases. Moreover, astrogliosis causes the formation of irreversible glia scarring that blocks axonal regeneration. One potential candidate that affects the behavior of reactive astrocytes is the transcription factor CCAAT/enhancer binding protein delta (CEBPD), which is responsive to inflammatory factors such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) in many inflammation-related diseases. To test the hypothesis that CEBPD modulates astrogliosis after SCI, we assessed a number of wound healing events using wild-type and CEBPD-knockout mice subjected to a moderate contusive injury at the mid-thoracic spinal level. We found that CEBPD was expressed by reactive astrocytes along the lesion border starting from 7 days after injury. CEBPD-deficient mice showed significantly reduced glial scar formation and increased residual white matter around the lesion compared with the wild-type mice 28 days after injury. In addition, CEBPD-deficient mice exhibited better recovery of motor function than the wild-type controls. Interestingly, CEBPD was associated with increased astrocytic expression of matrix metalloproteinase-3, suggesting a promotive role of CEBPD in astrocyte migration. Taken together, our results suggest that CEBPD may facilitate astrocyte migration and, thus, astrogliosis after SCI in mice.

Disclosures: S. Wang: None. **N. Chiu:** None. **J. Hsu:** None. **J. Wang:** None.

Poster

422. Spinal Cord Injury: Animal Models and Human Studies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 422.28/Y23

Topic: C.10. Trauma

Title: Spinal injury-induced spasticity in complete Th9 transection model in rats: Modulation by spinal glycine transporter 2 antisense oligonucleotide

Authors: *K. KAMIZATO[1], M. MARSALA[1], C. MAZUR[2], O. KAKINOHANA[1]
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Abstract: BACKGROUND: Imbalance between presynaptic excitation and inhibition after spinal injury plays a key role in the progressive increase in spinal reflexes and the appearance of spasticity. Glycine is a major inhibitory neurotransmitter in the central nervous system. The extracellular concentrations of glycine are regulated by Na+/Cl(-)-dependent glycine transporters (GlyTs), which are expressed in neurons (GlyT2) and adjacent glial cells (GlyT1). The goal of the present study was to assess the effect of GlyT2 inhibition on the spasticity response in animals with chronic transected spinal cord injury-induced muscle spasticity. METHODS: All animal studies were carried out under protocols approved by the Institutional Animal Care and Use Committee at UCSD. Adult Sprague-Dawley (SD) rats (female 200-300 g) had Th9 spinal segment transected to induce muscle spasticity. The presence of spasticity was defined as exacerbated EMG response recorded from the gastrocnemius muscle after applying progressively increased paw pressures using von Frey filaments (0.6-26 grams). After baseline spasticity measurement, animals received a single lumbar intrathecal bolus of GlyT2 antisense oligonucleotide (GlyT2-ASO;) or control antisense oligonucleotide (Cont-ASO). Before and after treatment the presence of spasticity response was measured in 1-week intervals for up to 4 weeks. RESULTS: In spastic animals receiving intrathecal injection of GlyT2-ASO, a progressive decrease in measured surface EMG activity after paw tactile stimulation was seen with the maximum effect measured at 2 weeks after injection ($p<0.05$). A significant anti-spastic affect was still present at 3 weeks after treatment, but was no longer present at 4 weeks. CONCLUSIONS: These data show that suppression of spinal GlyT2 activity may represent a novel therapy for modulation of chronic spinal injury-induced muscle spasticity.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 422.29/Y24

Topic: C.10. Trauma

Support: State of Florida, Department of Health

Title: Immune responses following transplantation of expanded autologous schwann cells for spinal cord injury in minipigs

Authors: *A. J. SANTAMARIA[1], F. D. BENAVIDES[1], L. G. GUADA[1], Y. NUNEZ[2], A. BROOKS[1], J. P. SOLANO[2], J. D. GUEST[1,3]
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Abstract: Introduction: Autologous Schwann cells (aSC) from sural nerves are being derived, expanded, and purified in cGMP compliant cell cultures and tested in a Phase 1 human clinical trial after spinal cord injury (SCI), [ClinicalTrials.gov Identifier: NCT01739023]. Preclinical studies used aSC in minipigs and primates, and either syngeneic SC in rodents or human SC + immune suppression in rodents. Here we report aSC transplants associated with substantial lymphocytic immune response in Yucatan minipigs and in one primate but not in rodent studies. Formation of lymphoid follicular structures has been reported in a mouse model after SCI, and in humans with MS but not following cell transplantation for SCI. Prior experiments utilized GFP-transfected cells except the rodent study with human cells. We thus tested two hypotheses: 1) the lymphocytic response is directed at the "foreign" green fluorescent protein. 2) ex-vivo expanded aSC are capable of MHC-restricted antigen presentation. Methods: To assess for the specificity of the lymphocytic response, female Yucatan minipigs underwent left hemi-contusion injuries at T6, T9 and T12. A nerve was extracted to derive aSC, each animal's preparation was divided into a GFP-transfected and non-transfected preparation. The cells were prepared in a manner similar to that for the clinical trial. The aSC were cryopreserved at passage 1 and later thawed and expanded to passage 2 for transplantation directly into the injury cavities 7 weeks after SCI: T6-non-transfected aSC, T12-GFP-transfected cells, T9-only a pial penetration was made, no cells were delivered. The concentration was 100,000 cells/µl and the volume delivered that which filled the injury cavity. Two months after transplantation, the animals were perfused. Results: In prior cohorts we observed the presence of perivascular lymphocytic infiltration as early as 1 day post-transplant and this response was only identified in 1/12 non-transplanted control animals. The injury sites occupied by transplanted aSC showed the formation of lymph-node like structures with germinal centers detected as early as 28d, and as late as 218d post-transplantation. CD3 positive lymphocytes were prominent in the paracortical and interfollicular zones of the formations. Staining for BCL-2 was used to exclude neoplastic growth. Transplanted aSC expressing myelin proteins were identified surrounding the nodal structures. Conclusions: Transplantation of ex-vivo purified aSC leads to a chronic organized lymphocytic response in the spinal cord of minipigs. It is important to determine the immunological mechanisms underlying this response and if they are unique to this species.

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Poster

422. Spinal Cord Injury: Animal Models and Human Studies

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 422.30/Y25

Topic: C.10. Trauma

Support: AOSpine

Title: Clinical and Surgical Predictors of Perioperative Complications in patients with degenerative cervical myelopathy: Results from the multicenter, prospective AOSpine International study on 479 patients

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Abstract: Introduction: Surgery for the treatment of cervical spondylotic myelopathy (CSM) is not without associated morbidity. By identifying important clinical and surgical predictors of complication development, clinicians can recognize their high-risk patients and institute appropriate prevention plans. This study was designed to identify important clinical and surgical predictors of perioperative complications in patients with degenerative cervical myelopathy. Material/Methods: Over a three-year period, 479 patients diagnosed with degenerative cervical myelopathy and treated surgically were enrolled in the prospective CSM-International study at sixteen global sites. A panel of physicians reviewed all adverse events and classified each one as related to CSM, related to surgery or unrelated. Univariate analyses were performed to determine demographic and surgical differences between patients who suffered a perioperative complication and those who did not. A final complication clinical prediction rule was developed using multiple logistic regression. Results: Eighty patients experienced 92 perioperative complications, yielding an incidence of 16.7%. Patients with complications were on average older (58.00±10.75) and had a higher BMI (26.62±4.50) compared to those without complications (56.04±12.12, 25.60±4.55), although these relationships did not reach significance. Univariately, the major clinical risk factors for perioperative complications were a diagnosis of OPLL, the number of comorbidities pre-operatively, diabetes, and co-existing gastrointestinal or cardiovascular disorders. There was no difference in the rate of complications between anterior or posterior approaches (p=0.744). Patients undergoing a 2-stage approach, however, were at a greater risk of perioperative complications than those treated with either

anterior or posterior surgery. Finally, patients with complications had a longer operative duration (198.41±92.30 min) than those without complications (173.98±77.02 min) (p=0.014). A final prediction model consisted of seven predictors: diabetes (OR=2.35), age (OR=1.02), operative duration (OR=1.003), two stage surgery (OR=20.37, p=0.012), OPLL (OR=1.82), gastrointestinal co-morbidities (OR=2.53) and body mass index (OR=1.06). Conclusion: Patients are at a higher risk of perioperative complications if they are older, have myelopathy secondary to OPLL, suffer from diabetes or gastrointestinal disorders, have a higher BMI and if they are treated by two-stage surgery or have a long operative duration.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.01/Y26

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA-JSTO

Title: Chemical warfare nerve agent induced gene expression alterations in blood: Indications for CNS-driven systemic injury

Authors: C. C. ROTHWELL[1], A. A. MELBER[1], C. S. HOFMANN[1], J. W. SEKOWSKI[2], *H. M. HOARD-FRUCHEY[1]
[1]USAMRICD, Aberdeen Proving Ground, MD; [2]US Army Edgewood Chem. Biol. Ctr., Aberdeen Proving Ground, MD

Abstract: Chemical warfare agents continue to be a persistent military and civilian concern on the battlefield and at home during the war against terrorism. Exposure to organophosphate chemical warfare agents such as soman, sarin, and VX results in alteration of cholinergic pathways. These agents bind to and disrupt the function of acetylcholinesterase, causing a variety of symptoms including miosis, excessive secretion, convulsions, seizure, and death. Although studies on the effects of chemical nerve agents began during the World War II era, the molecular mechanisms of these toxic agents are not well characterized. To characterize the systemic effects of VX at the molecular level, we used microarray analysis to identify gene expression changes in

whole blood samples. Adult male Sprague-Dawley rats were exposed to 0.4, 0.7, or 1.0 x LD_{50} VX (i.v.), and trunk blood was collected at 1, 2, 4, 8, or 24 h post-exposure. Total RNA was isolated and processed for mRNA and microRNA hybridization to Affymetrix GeneChip HT RG-230 PM and miRNA 3.1 array plates. For mRNA, principal component analysis (PCA) identified dose as the greatest source of variability within the dataset. Analysis of variance (ANOVA) was performed to identify nerve agent-induced changes in gene expression for 1 x LD_{50} VX over time. Using these data, pathways significantly affected were identified. Genes differentially expressed in response to VX exposure mapped to canonical pathways containing inflammatory and immunological molecules and molecules involved in cellular energy production. For microRNA, PCA identified time as the major source of variability within the dataset. Data analysis for the microRNA and integration with the mRNA dataset is ongoing. Characterizing the mRNA and miRNA expression changes within identified pathways may identify new systemic therapeutic targets for development of medical countermeasures as well as new biomarkers for diagnosis of chemical warfare nerve agent exposure. **Disclaimer:** The views expressed in this poster are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Edgewood Chemical Biological Center. This research was supported by the Defense Threat Reduction Agency - Joint Science and Technology Office.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: This research was supported by an interagency agreement between NIH/NIAID (AOD12058-001-00000, project #1) and the USAMRICD .

Title: Development of an aging rat model of nerve agent exposure for the evaluation of medical countermeasures

Authors: *M. C. MOFFETT, A. R. FURMAN, J. E. SCHWARTZ, M. F. STONE, G. E. GARCIA, L. A. LANGE
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Abstract: Older individuals are among one of the fastest growing populations in recent years and may be particularly vulnerable in the event of a terrorist attack using chemical weapon nerve agents. This project was designed to evaluate the susceptibility of aged rats to soman (GD) toxicity, including GD-induced seizures, neuropathology, and behavioral deficits. Male F344 rats 2 (adult) and 18 months (aged) of age were implanted with telemetry devices and exposed to GD (22-88 µg/kg). Whole blood cholinesterase was measured at multiple time points following GD exposure. Spatial memory acquisition was assessed using the Morris water maze (MWM) starting on post-exposure day (PED) 14. Brains were collected three weeks after exposure for pathological analysis. Aged rats showed a significantly greater sensitivity to the toxic effects of GD compared to adult rats. Although impairments in the MWM were observed in the aged compared to adult rats, there were no observed GD-induced spatial memory impairments following exposure. This was most likely due to the high mortality rate in the adult and aged animals that displayed EEG seizures. The majority of animals that survived to be tested in the MWM did not display seizures and did not have significant neuropathology. In the second experiment adult and aged rats were exposed to approximately equitoxic doses of GD (88 and 66 µg/kg respectively) and treated with atropine sulfate (2 mg/kg, im), the oxime HI-6 (93.6 mg/kg), and diazepam (10 mg/kg). Preliminary evaluation of medical countermeasures showed an effective reduction of mortality rates in both the adult and aged rats.

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Poster

423. Nerve Agents and Warfare Illness

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work was made possible by a CDMRP award (GW080094) to Dr. Fiona Crawford.

Title: Omic approaches identify lipid disturbances in mice exposed to Gulf War agents at a chronic 16 months post-exposure time-point

Authors: *L. ABDULLAH[1,2], J. EVANS[1], J. REED[1], G. CRYNEN[1], H. MONTAGUE[1], A. GONZALEZ[1], M. CROCKER[1], S. BAUMANN[3], Z. ZAKIRVA[1], T. EMMERICH[1], R. PELOT[1], G. AIT-GHEZALA[1,2], M. MULLAN[1], F. CRAWFORD[1,2]
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Abstract: Introduction: Gulf War Illness (GWI) continues to affect 25% of veterans who were deployment to the 1991 Gulf War (GW). The clinical presentation of this illness includes central nervous system (CNS) symptoms, such as cognitive problems and anxiety. To date, CNS pathobiology associated with chronic symptoms of GWI remains largely unknown, making it difficult to identify effective therapies. Therefore, identification of disturbed biological pathways associated with chronic GWI pathology is important for the development of appropriate treatments. Aim: Given the complex clinical presentation of GWI, we applied omic (lipidomics, proteomics and metabolomics) approaches to examine biological changes at 16-months post-exposure to GW agents. Methods: Nine-week old male C57BL6 mice were administered GW agents (pyridostigmine bromide [PB] and a pesticide permethrin [PER]) or control (DMSO) once daily for 10-days via intraperitoneal injections. Neurobehavioral testing was performed at 16-months post-exposure and then brain samples were collected for neuropathological and omic analyses. Lipidomics was performed using liquid chromatography/mass spectrometry (LC/MS) with in-source collision-induced dissociation on a LTQ mass spectrometer. Metabolomic analyses were conducted on trimethylsilyl derivatized samples by electron ionization gas chromatography (GC)/MS using a GC/QTOF mass spectrometer. Proteomic studies were performed using iTRAQ labeling with LC/MS/MS on a Q Exactive Orbitrap mass spectrometer. Results: Compared to controls, a significant decrease in several major phospholipid (PL) classes was observed in exposed mice. Ether PLs that are synthesized in peroxisomes were increased in exposed mice relative to controls. Metabolomic studies showed a decrease in free fatty acids in exposed mice vs. controls. Proteomic analyses showed that proteins that are involved in lipid degradation within lysosomes were modulated following exposure to GW agents. These omic changes corresponded with cognitive impairment and astroglia activation in the brains of exposed animals. Conclusion: These studies suggest an examination of peroxisomal and lysosomal function in laboratory models of GWI as likely candidates for novel targets to treat the CNS symptoms of GWI.

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Poster

423. Nerve Agents and Warfare Illness

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Program#/Poster#: 423.04/Y29

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant CA133483

Title: Testing for dose-dependent effects on object- and location memory following clinically relevant whole brain irradiation in adult rats

Authors: *D. R. RIDDLE[1,3], M. E. FORBES[1], M. PAITSEL[1], J. D. BOURLAND[2]
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Abstract: Whole brain irradiation (WBI) effectively treats brain tumors and metastases but many patients who survive > 6 months suffer cognitive decline due to normal tissue injury. The underlying mechanisms are not established and results of studies of brain irradiation in animal models are highly variable. We recently reported on a study of Fischer 344 x Brown Norway F1 rats irradiated at 3 or 17 mo-of-age with 40 Gy fractionated WBI (fWBI, 2 x 5 Gy/wk for 4 wk, BED 106.7 Gy) and then evaluated using novel object recognition (NOR) and novel object location (NOL) tasks from 3 to 12 mo after fWBI. These analyses revealed no effect on location memory but a radiation-induced, early-delayed deficit in object memory that was modest, transient and evident only in rats irradiated as young adults. Given the limited cognitive effects in that study, the mixed results of other studies, and recognition that rodents are more radioresistant than humans, another cohort of rats was treated at 3 mo-of-age with either sham irradiation, 40 Gy fWBI (8 x 5 Gy, BED 106.7), or 48 Gy fWBI (8 x 6 Gy, BED 144 Gy) and then evaluated longitudinally with the NOR and NOL from 1.5 to 12 mo after treatment. Analysis of discrimination ratios (DR) in the NOR with 6 min, 30 min, or 4 hr delay revealed no radiation-induced deficits at any time point following fWBI with 40- or 48 Gy. The average DR in the NOR 6 and NOR 30 remained stable in all groups from 1.5 to 12 mo after treatment, while the average DR in the NOR 4 hr declined in all groups starting at ~6 mo and was at chance levels by 12 mo. Measures of location memory from the NOL with 6 min and 4 hr delays were more variable than measures of object memory, were better with the shorter delay, and appeared unaffected by fWBI at either dose. Although novel object- and novel location discrimination appeared unaffected, fWBI altered some measures of activity during testing. The latency to begin exploring objects during the sample and test phases of the NOR was increased in rats receiving 40 Gy fWBI; surprisingly, rats receiving 48 Gy were more like sham irradiated control rats in

that measure. Irradiation with 40 but not 48 Gy also increased the average latency to enter the center zone of the testing arena during initial open field habituation at 4 wk after fWBI. Irradiation (regardless of dose) decreased the speed of movement during testing in the NOR, particularly in the first few mo after treatment. The results of this and previous studies of radiation-induced cognitive changes emphasize the need for continued investigation and validation of rodent models of radiation-induced brain injury, which are critical for developing new therapies for treatment-induced cognitive dysfunction in cancer survivors.

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Poster

423. Nerve Agents and Warfare Illness

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Radiation Safety Authority

EU, FP7-Fission-2011, CEREBRAD, GA no: 29555

Title: Cognitive defects and tau protein alterations in adult mice following neonatal low dose co-exposure to radiation and ketamine

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Abstract: Ionizing radiation (IR) is widely used in medicine for treating tumours, including tumours in the central nervous system, and for imaging techniques e.g. computed tomography (CT). Ketamine is an anaesthetic agent commonly used in children undergoing repeated anaesthesia in radiotherapy of tumours but also as sedative agent in CT diagnosis. Although its great benefits in medicine awareness has been raised about possible negative consequences from low dose exposure to IR during brain development and also the effects a co-exposure situation with IR and ketamine might have on the vulnerable developing brain. In previous studies we have indicated that IR as well as ketamine can induce persistent neurotoxic effects in mice, manifested as reduced cognitive function and altered levels of the neuroproteins CaMKII, GAP-

43 and tau, when exposure occurs during a defined critical phase of brain development. The present study was conducted to explore developmental neurobehavioural and neuroprotein effects in mice co-exposed to IR and ketamine during neonatal brain development. Male neonatal NMRI mice were exposed to a single 7.5 mg/kg b.w. dose of ketamine s.c. on postnatal day 10. One hour following ketamine exposure the mice were placed in plastic dishes and irradiated to a single dose of 50, 100 or 200 mGy from a 137Cs source at a dose rate of 0.2 Gy/min (Rudbeck laboratory, Uppsala University). Mice were also exposed to only ketamine or IR for equivalent doses as co-exposed mice. Control animals were exposed to 0.9% saline (10 mg/kg b.w.) s.c. or placed in plastic dishes and sham irradiated. At 2- and 4-months of age the animals were tested for spontaneous behaviour in a novel home environment. At 5-months of age the animals were tested in a Morris water maze (MWM). Two weeks after the MWM mice were euthanized and dissected for further analyses of proteins CaMKII, GAP-43, synaptophysin and tau in cerebral cortex and hippocampus. Animals co-exposed to IR and ketamine developed an altered cognitive function, manifested as a lack of habituation capacity, in a dose-response related manner. In the MWM animals co-exposed to IR and ketamine showed an impaired memory and learning capacity. Furthermore, in mice co-exposed to IR and ketamine and showing reduced cognitive function in the behavioural tests levels of tau protein was increased in cerebral cortex. This study shows that co-exposure to IR and ketamine can interact and aggravate developmental neurotoxic effects manifested as impaired habituation, learning and memory ability, indicating reduced cognitive function, in a dose-response related manner and at doses where the sole agents alone don't have any impact on the measured variables.

Disclosures: S. Buratovic: None. **B. Stenerlöw:** None. **A. Fredriksson:** None. **S. Sundell-Bergman:** None. **P. Eriksson:** None.

Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.06/Y31

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Reduced carbachol-induced β/γ oscillations in CA3 region of hippocampus after post-natal contamination of uranium in adult rat

Authors: *C. DINOCOURT, J. STEFANI, C. ELIE, P. LESTAEVEL, I. DUBLINEAU, P. GOURMELON
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Abstract: During period of brain development, heavy metals can induce developmental neurotoxic effects. Uranium (U) is naturally present in the environment since it is a component of the earth's crust. Civilian and military industries increase U concentration in the soil and underground water. Therefore populations may be chronically exposed by ingestion of drinking water or food. Previous studies have shown that U is present in brain and alters behavior. In young rat exposed to U from birth through drinking water, object recognition memory was decreased and was associated with a reduction of ACh concentration and AChE activity in the entorhinal cortex. The objective of this study is to assess whether gamma range oscillations induced by cholinergic agonist could be involved in the deficit of learning and memory. Synchronous network activity was recorded in the hippocampus of rats contaminated from birth during 6 months by depleted uranium (DU) at 10 and 40mg/l and non-contaminated rats. We analyzed β/γ oscillations (20-80Hz) induced by the cholinergic agonist, carbachol (CCH) in CA3 region of hippocampus slices. Bath application of CCH (20μM) generates fast rhythmic activity with a frequency in β range and a secondary in the γ range in slices from non-contaminated as well as contaminated animals. The peak frequency of these oscillations is not different between non-contaminated and contaminated animals. However, our results show less powerful of β range oscillations in animals contaminated at 40mg/l compared to control animals. Indeed, none of the slices from animals contaminated at DU40 displays a power higher than 50μV2/Hz. In contrast, 83% of slices from control rats and 83% of slices from rats contaminated at DU10 show a power higher than 50μV2/Hz. Since fast-spiking interneurons are necessary for the generation of γ range oscillatory synchronization, analysis of parvalbumin-containing interneurons has been investigated. Number of parvalbumin-positive cell bodies is not reduced in CA3 region. No difference has been observed in the distribution of synaptic terminals of these interneurons in contaminated animals compared to control animals. In conclusion, these results show a decrease of gamma range oscillations without change of peri-somatic interneuron network. These suggest that synchronous oscillations could be disturb by chronic exposure of U and thus could result in cognitive deficits. Further experiments have to be performed, especially on basal neurotransmission and excitability as well as interneuron network.

Disclosures: C. Dinocourt: None. **J. Stefani:** None. **C. Elie:** None. **P. Lestaevel:** None. **I. Dublineau:** None. **P. Gourmelon:** None.

Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.07/Y32

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Is neurogenesis altered after chronic internal contamination of uranium during brain development?

Authors: *M. LEGRAND[1], C. IBANEZ[1], P. LESTAEVEL[1], J. STEFANI[1], N. FLORES[1], P. ERIKSSON[2], C. DINOCOURT[1]
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Abstract: Uranium (U) is a heavy metal naturally found in the environment. Its many uses in civil or military technologies give cause of concern about human health risks. A lot of studies highlight the effects of uranium on cerebral functions such as cognitive tasks. For example, contamination via drinking water in adult rats induced spatial memory impairment and sleep/wake cycle disturbance. Decrease of object recognition memory has also been showed after post-natal exposure to U. Neurogenesis is known to be involved in learning and memory defects. Therefore, we analysed the effects of depleted uranium (DU) on neurogenesis during brain development in an *in utero* model of internal contamination. Pregnant rats were contaminated by drinking water at DU 40 and 120 mg/L during gestation and lactation. At embryonic stages (E13, E18) and post-natal stages (PND 0, PND 21) we examined the structural morphology of the brain by Nissl staining, the cell proliferation by BrdU immunostaining and the cell death by Fluorojade staining. Our results show that DU does not generate major organogenesis deficits in the telencephalon of embryos and in the dentate gyrus of young rats. In animals contaminated by U at 40mg/L, no significant change in cell proliferation and cell death is observed at each time point. However, these processes are impaired in animals contaminated by U at 120mg/L. At E13, results show a decrease of BrdU staining in cortical neuroepithelium in contaminated embryos without modification in cell death. In contrast, at E18, an increase of BrdU staining is observed in hippocampal neuroepithelium. At this stage, results also show a decrease of fluorojade-positive cells in cortical neuroepithelium. At PND 0, the number of Fluorojade-positive cells decreases in the dentate gyrus but no modification of cell proliferation was noted. Finally at PND 21, quantitative analysis shows a decrease of BrdU positive cells in the dentate gyrus of hippocampus of contaminated rats. In conclusion, these results show that *in utero* contamination by U leads to changes in cell proliferation and cell death processes during brain development. These could have an impact in the next steps of neurogenesis (differentiation, migration, synaptic connectivity) and could consequently result in defects on the organization of neuronal network. So, these results suggest that chronic exposure to U in embryonic life could lead to behavior disorders in adulthood. More experiments have to be performed to study the other processes of neurogenesis such as gene and protein expressions of specific markers of differentiation and migration.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.08/Z1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

Title: The effect of aging on neural stem / progenitor cell populations after radiation

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Abstract: Aging is associated with decreased neurogenesis in the dentate gyrus (DG), and cranial radiation treatment (RT) is known to ablates DG neurogenesis. Aging may further compound the development of severe and irreversible cognitive dysfunction after RT. Inhibition of neurogenesis is considered to be an important underlying mechanism of RT-associated cognitive deficits. It has been reported that the extent of post-RT reduction in neurogenesis is diminished with aging. Here, we investigated the influence of aging on changes in neural stem cells and neural progenitor cells (NPCs) in DG after RT. METHODS Male mice aged 2, 4, 6, 12, and 18 months old were given 0 or 5 Gy. Newborn cells were labeled with a bromodeoxyuridine (BrdU) incorporation assay 4 weeks post-RT. The brains were harvested and fixed 4 weeks after BrdU. The newborn neurons, type I (putative neural stem cells), and type II cells in the DG were identified using immunohistochemistry with specific phenotypic markers and estimated by stereological methods. Two-way ANOVA was used to analyze the results. RESULTS In the DG, the total number of newborn cells (BrdU+) decreased with aging ($p < 0.0001$; 448 ± 89 at 2 months, 8.5 ± 8.5 at 18 months) and after RT ($p = 0.0007$; 68% reduction at 2 months, 33% reduction at 18 months). The number of newborn neurons decreased with aging ($p < 0.0001$; 191 ± 88 at 2 months, 4.5 ± 4.5 at 18 months) and after RT ($p < 0.0001$, 53%, 43%, 60%, 14%, and 100% reduction at 2, 4, 6, 12 and 18 months respectively). The interaction between age and RT was significant ($p = 0.0019$). The number of newborn type I ($p = 0.0001$; 90 ± 18 at 2 months, 8.5 ± 8.5 at 18 months), total type I ($p = 0.002$; 8271 ± 120 at 2 months, 4493 ± 592 at 18 months), and total type II cells ($p = 0.001$; 472 ± 90 at 2 months, 280 ± 14 at 18 months)

decreased with aging. RT decreased the number of total type I cells ($p = 0.01$; 33% reduction at 2 and 18 months). RT did not have a significant influence on the number of newborn type I and total type II cells when adjusted for age. CONCLUSION The age-related decline in neural stem cells and NPCs may contribute to the age-related decline in newborn neurons. The loss of putative neural stem cells after RT may contribute to the post-RT decline in neurogenesis, and the extent of post-RT decline in neural stem cells and newborn neurons do not seem to be more severe with aging.

Disclosures: Z. Cheng: None. **Y. Li:** None. **S. Wong:** None.

Poster

423. Nerve Agents and Warfare Illness

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Program#/Poster#: 423.09/Z2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Congressionally Directed Medical Research Program Grant #:GW100076

Title: Anatabine ameliorates cognitive impairment and neuropathological deficits in a mouse model of Gulf War Illness

Authors: *G. AIT-GHEZALA[1,2,3], Z. ZAKIROVA[1,2,3], B. MOUZON[1,2,3], M. TWEED[1,2,3], D. PARIS[1,2,3], V. MATHURA[1,2], F. CRAWFORD[1,2,3], M. MULLAN[1,4]
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Abstract: Gulf War Illness (GWI) is a multisymptom chronic illness with a central nervous system (CNS) component, symptoms of which include cognitive impairment such as memory and neurological deficits, fatigue, and musculoskeletal problems, as well as immune and inflammatory dysfunction, as key consequences of exposure post deployment to the Persian Gulf War. There are ample data that suggest that exposure to Gulf War (GW) agents, such as pyridostigmine bromide (PB) and pesticides such as permethrin (PER), were key contributors to the etiology of GWI. We have previously demonstrated that C57BL6/J mice at 5 months post exposure to GW agents early in life develop late-onset cognitive impairment, and neuropathological changes marked by an elevation of neuroinflammatory markers. This model of chronic consequences of acute exposure to GW agents is thus relevant to the veterans currently

suffering with GWI as their exposure occurred over 20 years ago. We have previously shown *in vitro* and *in vivo* the efficacious anti-inflammatory properties of Anatabine, a minor tobacco alkaloid also present in plants of the Solanacea family, which displays a chemical structural similarity with nicotine (Paris et al., 2013). In the current study we investigated whether daily oral administration of Anatabine (2o mg/kg) could be used to mitigate neuroinflammation, and ameliorate cognitive impairment and neuropathological deficits observed in our mouse model of GWI. Behavioral analyses of learning and memory were performed in control and GW agent exposed mice at 5 months post-exposure, at the end of behavior assessment, a treatment or placebo began and the mice were assessed again 1 and 2 months post treatment with Anatabine. Our results demonstrate that 2 months post treatment with Anatabine we were able to rescue the neurobehavioral deficits we previously observed in these mice. Immunohistochemical (IHC) analyses are ongoing in brain sections from these animals. Thus, Anatabine is a promising potential therapeutic that may be used alleviate some of the symptoms experienced by GW veterans today. Possible competing interests: MM is the CEO of Rock Creek Pharmaceuticals which sells anatabine as a nutraceutical supplement. The anatabine used in this study was provided by Rock Creek Pharmaceuticals. None of the other authors receive remuneration from Rock Creek Pharmaceuticals. This study was funded by a CDMRP Grant to GAG.

Disclosures: **G. Ait-Ghezala:** None. **Z. Zakirova:** None. **B. Mouzon:** None. **M. Tweed:** None. **V. Mathura:** None. **F. Crawford:** None. **M. Mullan:** A. Employment/Salary (full or part-time):; Rock Creek Pharmaceuticals, Sarasota, Florida, USA.. **D. Paris:** None.

Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.10/Z3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: SBIR Phase 2 award #4R44NS068049-02

Title: Galantamine as an effective pre-treatment countermeasure against high doses of soman: Comparison with pyridostigmine efficacy

Authors: *Y. ARACAVA[1], E. F. R. PEREIRA[1], M. LANE[1], R. J. CLARK[1], G. W. BASINGER, Jr.[2], E. X. ALBUQUERQUE[1]
[1]Div. of Translational Toxicology, Dept Epidemiol Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD; [2]Countervail Corp., Charlotte, NC

Abstract: Organophosphorus (OP) nerve agents have resurged recently as deadly weapons used in Middle East conflicts and as means to spread terror among civilians. By irreversibly inhibiting acetylcholinesterase (AChE), they lead to life-threatening cardio-respiratory arrest and status epilepticus-like seizures. It is known that the toxicity of the nerve agent soman cannot be readily counteracted by the available treatments, especially when dealing with doses higher than 2xLD50. Here, we compared the effectiveness of pre-treatment with galantamine vs. pyridostigmine against 2x and 3xLD50 soman in both male and female guinea pigs. Thirty min prior to the s.c. injection of soman, guinea pigs were treated i.m. with galantamine, pyridostigmine or saline. Post-soman treatment consisted of the muscarinic receptor antagonist atropine (0.5 mg/kg) and the AChE reactivator 2 PAM (25 mg/kg), both given at 1 min post-soman, and midazolam (2.2-2.8 mg/kg) administered 5 min post-seizure onset. Animals challenged with 2xLD50 soman received one post-treatment cocktail, whereas those challenged with 3xLD50 received the post-treatment cocktail three times at 1.5 h intervals. At 24 h post-soman injection, we evaluated the effectiveness of the treatments to prevent lethality, blood and brain AChE inhibition, and neurodegeneration (assessed with Fluoro-Jade B, FJ-B, staining). Against 2xLD50, pre-treatment with galantamine (6-10 mg/kg) afforded 100% protection from lethality; 71% and 0 % of the animals survived when pre-treated with pyridostigmine (0.026 mg/kg) and saline, respectively. Against 3xLD50, pre-treatment with 8, 10 and 12 mg/kg galantamine afforded 67, 71 and 100% survival, respectively, whereas pre-treatment with pyridostigmine and saline resulted in 38% and 14% survival, respectively. Clinical signs of intoxication had slower onset, lower severity, and shorter duration in galantamine- than in pyridostigmine- or saline-pretreated animals. Large numbers of FJ-B-positive cells were seen in numerous brain regions of most of the pyridostigmine- and saline-pretreated guinea pigs, whereas cell death was absent in the brains of soman-injected guinea pigs that had been pre-treated with galantamine. Finally, brain and blood AChE activity were more preserved among soman-injected rats that had been pre-treated with galantamine than among those pre-treated with pyridostigmine or saline. Thus, our results reveal that lethality and neurotoxicity induced by supra-lethal doses of soman is effectively counteracted by pre-treatment with galantamine in conjunction with post-treatment with atropine, 2-PAM, and diazepam.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Funded in whole or in part with Federal funds from the Biomedical Advanced Res & Devel Authority, Office Asst Secretary for Preparedness & Response, Office Secretary Dept Health & Human Services, under Countervail Corp Contr #: HHSO100201100030C

Title: Effects of galantamine post-treatment on nerve agent-induced EEG changes and lethality of rats

Authors: *E. A. ALEXANDROVA, Y. ARACAVA, J. D. PESCRILLE, L. D. RICHARDSON, B. GUSHEN, E. F. R. PEREIRA, E. X. ALBUQUERQUE
Div. of Translational Toxicology, Dept. Epidemiology & Publ. Hlth., Univ. Maryland Sch. of Med., Baltimore, MD

Abstract: Control of seizure activity resulting from overactivation of the brain cholinergic system due to irreversible acetylcholinesterase (AChE) inhibition by the organophosphorus (OP) nerve agents sarin and VX is essential to improve survival and limit brain damage. Here, we analyzed cortical EEG recordings from rats exposed to 1.2xLD50 sarin or VX to determine if inclusion of galantamine in a post-OP exposure treatment regimen consisting of the muscarinic receptor antagonist atropine and the AChE reactivator 2-PAM reduces OP-induced seizures and lethality. To this end, cortical EEG was telemetrically recorded for 24 h from rats injected with 1.2xLD50 of sarin or VX and immediately post-treated with atropine (0.5 mg/kg)-plus-2-PAM (25 mg/kg) and galantamine (0.3, 1.0, or 3.0 mg/kg) or saline. After the treatment with atropine, 2-PAM, and saline, 73% of the sarin-injected rats survived and 54.5% developed seizures. The total EEG power of the convulsing rats increased significantly, primarily due to a nearly 50-fold increase in the power of the low-frequency delta band (0.5-4 Hz); the power of the high-frequency gamma band (32-50 Hz) increased only 12 times during first hour of seizures and then gradually decreased to 68% below baseline at the end of experiments. In the EEG of sarin-injected rats that did not convulse, the total power and the delta power density were decreased (40-45%) shortly after the sarin challenge. At the same time, the gamma band power increased up to 85% in the first 3 h after injections. The EEG and survival of sarin-challenged rats that were post-treated with 0.3 or 1 mg/kg galantamine were comparable to those of rats that had been post-treated with saline. Increasing the dose of galantamine to 3 mg/kg increased survival of sarin-injected rats to 88% and significantly reduced to 12% the incidence of seizures. In this group, the gamma band power density remained 62% above the baseline level at 20 h after sarin. All animals that were challenged with VX and post-treated with atropine, 2-PAM, and saline survived and only 28% developed short periods of seizures. In non-convulsing rats, VX induced more profound changes than sarin in the power density of the delta (~65% decrease) and gamma (~2.2-fold increase) bands. Inclusion of galantamine in the post-treatment regimen reduced to 17% the incidence of seizures among VX-challenged rats. These results demonstrate a strong

relationship between seizure occurrence and the simultaneous changes of energy in gamma and delta bands shorty after OP exposure. They also reveal that galantamine associated with atropine and pralidoxime effectively protects animals against OP-induced seizure and lethality.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.12/Z5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant ES019282

Title: Prenatal exposure to Chlorpyrifos leads to reduced msk expression in the dentate gyrus: Implications for regulation of histone acetylation

Authors: ***S. W. TODD**[1], W. R. RANDALL[2], E. F. R. PEREIRA[1], E. X. ALBUQUERQUE[1]
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Abstract: Chlorpyrifos (CPF) is an organophosphorous insecticide that is used worldwide. Sub-acute exposure to CPF during pregnancy has been correlated with increased incidence of cognitive dysfunction in children [Neurotoxicol Teratol 34:534, 2012]. While the acute toxicity of CPF results primarily from its ability to irreversibly inhibit acetylcholinesterase (AChE), the enzyme that hydrolyzes the neurotransmitter acetylcholine, the mechanisms underlying the neurological deficits induced by prenatal exposure to sub-acute levels of CPF are poorly understood. Recent studies have demonstrated that (i) decreased histone acetylation in the hippocampus reduces the activation of genes that are related to cognitive function [Nature 447:178, 2007] and (ii) injection in the hippocampus of a drug that increases histone acetylation improves cognition in a mouse model of Alzheimer's disease [Science 328: 753, 2010]. These findings relate well to studies that identify histone acetylation as a requirement for the formation of fear memory [Cell 156:261, 2014]. The present study was designed to test the hypothesis that prenatal exposure to sub-acute levels of CPF leads to a decreased expression of mitogen- and stress- activated kinase (MSK) protein as well as a reduction in the levels of acetylated histones in the whole hippocampus. Among many substrates, MSK is a primary activator cAMP

Response Element Binding (CREB) protein, which can directly acetylate histone residues [Mol Cell Biol 21:476, 2001] and as a result, increase the expression of genes needed for memory consolidation [Neuropsychopharmacology Rev 38:62, 2013]. To test this hypothesis we exposed guinea pigs to CPF (25 mg/kg/d, sc) or peanut oil during the final 10 days of pregnancy. On postnatal day 80, brains were harvested from offspring and processed for western blots and immunohistochemistry. Total MSK protein expression in the dentate gyrus via immunohistochemistry and levels of acetylated histone H3 in the whole hippocampus via western blot were significantly decreased in male guinea pigs that had been prenatally exposed to CPF. Disruption of the epigenome may contribute to the neurological deficits observed in those who have been developmentally exposed to sub-acute levels of CPF.

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Poster

423. Nerve Agents and Warfare Illness

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Program#/Poster#: 423.13/Z6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant ES019282

Title: *In utero* exposure to the pesticide chlorpyrifos leads to augmented gabaergic synaptic transmission and gliosis in the guinea pig hippocampus

Authors: *R. D. BURKE, E. X. ALBUQUERQUE, E. F. R. PEREIRA
Div. of Translational Toxicology, Dept. of Epidemiology and Publ. Hlth., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Epidemiological studies have reported that the incidence of cognitive deficits is markedly high among children exposed *in utero* to organophosphorus pesticides, including chlorpyrifos (CPF) [Neurotoxicol Teratol 34:534, 20121]. Studies of animal models have suggested that cognitive impairments induced by prenatal exposure to CPF may result from disruption of the functional integrity of the hippocampus [Brain Res 1474:19, 2012]. Because increased GABAergic activity is known to induce cognitive impairments [Psychopharmacology 145:213, 1999], we hypothesized that GABAergic transmission in the CA1 field of the hippocampus increases as a result of prenatal exposure to CPF. To test this hypothesis, we

recorded inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) from CA1 pyramidal neurons in hippocampal slices from adult male guinea pigs that were born to dams injected with CPF (25 mg/kg/day) or vehicle (peanut oil) during the last ten days of gestation. This CPF dose regimen caused no overt signs of maternal toxicity and resulted in ca. 40% red blood cell acetylcholinesterase inhibition in newborn guinea pigs. Spontaneous and miniature IPSC frequencies were higher in CPF-exposed than in control guinea pigs, while spontaneous and miniature EPSCs were comparable between treatment groups. Western blotting performed on protein extracts from the hippocampi of guinea pigs used in the electrophysiological experiments revealed that prenatal exposure to CPF had no significant effect on the expression of cellular markers of specific interneuron populations and reduced the expression of glutamic acid decarboxylase, an enzyme that catalyzes GABA synthesis. As such, the enhanced GABAergic transmission in the hippocampus of CPF-exposed animals is unlikely due to an increased number of interneurons and/or increased synthesis of GABA. However, expression of glial fibrillary acidic protein, an astrocyte marker, was significantly increased in the hippocampus of the guinea pigs that had been prenatally exposed to CPF. Given earlier reports that astrocytes promote the development of inhibitory synapses in the hippocampus [J Neurosci 25:3638, 2005], it is tempting to speculate that gliosis takes place and leads to an increase in GABAergic transmission that, in turn, contributes to the cognitive impairments observed later in life following prenatal exposure to CPF.

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Poster

423. Nerve Agents and Warfare Illness

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Program#/Poster#: 423.14/Z7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Corticosterone enhances chlorpyrifos-induced neuroinflammation

Authors: *J. P. O'CALLAGHAN[1], K. A. KELLY[1], D. B. MILLER[1], S. M. LASLEY[2]
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Abstract: Elevated expression of proinflammatory mediators in the CNS serves as the basis for normal and pathophysiological neuroinflammation. For example, the enhanced expression of proinflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-6 and CCL2 may underlie

the general malaise termed sickness behavior as well as depression and can affect and contribute to a risk of neurodegeneration in affected individuals. Chemical exposures that increase the degree or duration of proinflammatory responses in the CNS may lead to more severe symptoms and consequences of neuroinflammation. Our prior data indicate that acute exposure of the C57Bl6/J mouse to the irreversible cholinesterase inhibitor, diisopropylfluorophosphate (DFP), results in a neuroinflammatory response in multiple brain regions, as evidenced by enhanced expression of a large variety of proinflammatory cytokines over a 12-hr post exposure period. Surprisingly, treatment with anti-inflammatory glucocorticoid, corticosterone (CORT), enhanced rather than suppressed DFP-induced neuroinflammation. These findings suggested that CORT might serve as a stressor surrogate to "prime" the neuroinflammatory response to workplace and environmentally relevant exposures to organophosphates, such as the widely used pesticide, chlorpyrifos (CPF). Here, we administered CPF (8 mg/kg, i.p.) with and without prior CORT (400 μg/ml in the drinking water for 4 days) to C57Bl6/J male mice. As with DFP, CPF alone caused neuroinflammation (increases in mRNA for TNF-α, LIF, CCL2 and OSM) at 6 hrs. post dosing. These effects were markedly enhanced by the prior exposure to CORT and broadened to include another cytokine (IL-1β). These findings suggest that stressors experienced by pesticide applicators and other workers exposed to CPF will contribute to a neuroinflammatory response of yet to be characterized duration or severity. (supported by intramural funds from CDC-NIOSH)

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Poster

423. Nerve Agents and Warfare Illness

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Title: The pharmacokinetics of galantamine in adult male rats is not affected by the organophosphorus nerve agent sarin or by atropine, pralidoxime, and diazepam

Authors: W. P. FAWCETT[1], R. H. COOMBES[1], Y. ARACAVA[1], E. J. WAKAYAMA[2], G. W. BASINGER, Jr[3], E. X. ALBUQUERQUE[1], *E. F. PEREIRA[1]
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Abstract: Galantamine, a reversible acetylcholinesterase inhibitor currently approved for treatment of Alzheimer's disease, has emerged as an effective antidote against the toxicity of organophosphorus (OP) nerve agents, including sarin (Albuquerque et al., PNAS 103: 13220, 2006). Here, we analyzed the pharmacokinetic profile of galantamine in young adult male rats that received the following treatments: (i) galantamine (1, 3, or 6 mg/kg, im); (ii) atropine (0.5 mg/kg, im)-plus-pralidoxime (25 mg/kg, im) followed 10 min later by diazepam (0.072 mg/kg, im) and galantamine (1, 3, or 6 mg/kg); or (iii) 0.8xLD50 sarin followed 1 min later by atropine-plus-pralidoxime and 10 min later by diazepam and galantamine (3 mg/kg). Blood was collected at various time points from a jugular vein catheter into heparinized ependorf tubes. Plasma concentrations of galantamine were determined by means of high-performance liquid chromatography as described in Albuquerque et al. (2006). The area under the plasma concentration vs. time curve (AUC), half-life (1/2), maximum plasma concentration of galantamine (Cmax), and time to reach Cmax (Tmax) were evaluated through noncompartmental pharmacokinetic analysis using the software Kinetica 5.0 (Adept Scientific Ltd, Herts, UK). Table 1 shows the values of these parameters for each dose of galantamine. Treatment of the rats with drugs used in the standard care of OP intoxication, specifically the muscarinic receptor antagonist atropine, the cholinesterase reactivator pralidoxime, and the anticonvulsant diazepam, had no significant effect on the pharmacokinetics of galantamine. Likewise, the pharmacokinetics of galantamine was not affected by exposure of the rats to 0.8xLD50 sarin. These findings are far-reaching for the evaluation of galantamine as a potential neuroprotecting agent against the immediate and delayed toxicity of sarin, as they demonstrate that the *in vivo* disposition of galantamine is not affected by ancillary standard-care therapies normally used to halt the acute cholinergic crisis induced by sarin or by sarin itself.

	Galantamine (mg/kg, im)		
	1	3	6
Cmax (µg/ml)	0.41 ± 0.10	0.92 ± 0.28	1.78 ± 0.55
Tmax (min)	5.92 ± 3.02	8.33 ± 4.92	10.5 ± 5.22
AUC (µg/ml.min)	29.7 ± 6.43	58.3 ± 12.8	133.6 ± 33.0
t1/2 (min)	51.2 ± 27.7	63.2 ± 27.3	77.2 ± 24.2

Table 1. Pharmacokinetics of galantamine in adult male rats. Data are mean ± SD of results from 10 animals per treatment.

Disclosures: W.P. Fawcett: None. **R.H. Coombes:** None. **Y. Aracava:** None. **E.J. Wakayama:** None. **G.W. Basinger:** None. **E.X. Albuquerque:** None. **E.F. Pereira:** None.

Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.16/Z9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant ES019282

Title: Long-lasting histone acetylation of pyramidal neurons are detected in the hippocampus of young adult guinea pigs exposed *in utero* to the organophosphorus pesticide chlorpyrifos

Authors: *W. R. RANDALL[1], E. F. R. PEREIRA[2], E. X. ALBUQUERQUE[2]
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Abstract: Abstract: Organophosphorus (OP) pesticides such as chlorpyrifos (CPF) and malathion are among the most heavily used pest control agents worldwide. Epidemiological data indicate that OP pesticides are toxic to the developing human brain. In-utero exposure to OP pesticides has been linked to delayed mental development in young children as well as autism spectrum disorders and attention deficit hyperactivity. Although acute toxicity of OP pesticides results primarily from the rapid inhibition of acetylcholinesterase, the delayed toxicity is more likely related to long-term changes in gene expression. We hypothesize that *in utero* exposure to CPF alters epigenetic mechanisms, particularly histone modifications, that induce changes in the expression of genes known to control higher brain functions. To test this hypothesis, we used guinea pigs, a precocious rodent that mimics many characteristics of human OP intoxication. Pregnant guinea pigs were injected subcutaneously once daily with 25 mg/kg CPF or with peanut oil (the CPF vehicle) for 10 days starting on gestation day 50-53. Guinea pigs generally delivered their pups at GD65-67. On post-natal day 80, animals were euthanized and their brains were removed and processed for immunohistochemistry using antibodies to acetylated histones and histone deacetylase2 (HDAC2). Levels of acetylated histone H2B and acetylated histone H3 were altered in pyramidal cells of the hippocampal CA1 region in animals developmentally exposed to CPF *in utero* than in CA1 pyramidal cells of the controls whose mothers were given peanut oil diluent. Previous studies indicated that the expression of p38 kinase was significantly higher in the brains of animals developmentally exposed to CPF than in the brains of control animals. This is noteworthy because p38 is one of the kinases that activates mitogen- and stress-activated kinases 1 and 2 (MSK1/2), which have been identified as a principal histone H3 kinase and in mediating histone H3 acetylation. Together these results suggest that exposure to CPF during early development produces epigenetic modifications in the acetylation of histones H2B

and H3, possibly though activation of histone H3 acetylase through p38 signaling that could lead to long-term changes in gene expression accompanying the neuronal abnormalities observed in the deficits in cognition.

Disclosures: W.R. Randall: None. **E.F.R. Pereira:** None. **E.X. Albuquerque:** None.

Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.17/Z10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: Comparison of brain injury following soman exposure by inhalation and subcutaneous injection

Authors: *R. K. KAN, J. A. LEUSCHNER, B. J. WONG, M. W. PERKINS, T. L. DAO, J. L. DEVORAK, L. J. SHUMWAY, A. M. RODRIGUEZ, A. M. SCIUTO
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Abstract: The most likely route of exposure to the chemical warfare nerve agents (CWNAs) is inhalation. Animals subcutaneously injected to a convulsive dose of the CWNA soman developed severe brain damage in different regions of the limbic structure. The present study was designed to compare the magnitude of brain injury following inhalation and subcutaneous exposure to the CWNA soman. Untreated, non-anesthetized rats were exposed to 600 mg X min/m^3 of soman vapor containing perfluorohexane (PFH). Control rats were exposed to PFH. Rats designated for subcutaneous exposure were pretreated with HI-6 (125 mg/kg, ip) 30 min prior to soman challenge (180 ug/kg, sc). At 1 min after soman injection, rats were given atropine methylnitrate (AMN; 2.0 mg/kg, im). HI-6 and AMN were given to increase survival without affecting the development of convulsions. Control rats received HI-6, saline instead of soman and AMN. All 24 hr survivors were anesthetized and perfused with saline, followed by 10% formalin. Brains were cut into 3 mm slices and then processed in paraffin. Serial sections were cut at 5 μm and then stained with hematoxylin and eosin for neuropathological evaluation. Rats that developed convulsions after inhalation and subcutaneous exposure had severe brain injury in the piriform cortex, amygdala, thalamus, dentate hilus and neocortex. Rats that did not convulse were free of neuropathology. The results indicate that regardless of route of exposure,

soman causes convulsions and brain injury. In addition, our results solidify the notion that convulsions are a primary pathogenic factor of brain injury since no brain injury was observed in non-convulsing rats. Both exposure routes are suitable for studying CWNA-induced neurological toxicity and brain injury and evaluating potential countermeasures.

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Poster

423. Nerve Agents and Warfare Illness

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 423.18/Z11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: MicroRNA expression profiling reveals mRNA regulating specific signaling pathways in ventral hippocampus following nerve agent exposure

Authors: *J. M. PIZARRO[1], T. L. DAO[2], J. A. LEUSCHNER[2], S. W. KASKI[2], L. J. SHUMWAY[2], C. R. BRAUE[2], R. K. KAN[2]
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Abstract: The ventral hippocampus has been shown to be more susceptible to seizure and nerve agent-induced neuropathology than the dorsal hippocampus. Therefore, the ventral hippocampus is the ideal brain region in which to study the role of microRNA and identify the microRNA-mRNA signature involved in soman-induced brain injury. Rats were pretreated with HI-6 (125 mg/kg, ip) 30 min prior to soman injection (180 ug/kg, sc). At 1 min after soman challenge, rats were given atropine methylnitrate (2.0 mg/kg, im) to reduce peripheral toxicity. At 1 hr, 3 hrs, 6 hrs, 12 hrs and 24 hrs after the onset of convulsions, rats were anesthetized, and the ventral hippocampi were dissected for investigating mRNA targets of differentially expressed miRNAs. The results showed an upregulation of a distinct set of miRNAs at different times after the onset of soman-induced convulsions. Among the significantly differentially expressed miRNAs, miR-16, miR-21, miR-29b, miR-30e, miR-34a, miR-124, miR-135b, miR-140, miR-142-3p, miR-181c, miR-212, miR-215, miR-411, and miR-503 were upregulated and are associated with the

regulation of neuronal death. In addition, a number of miRNA target genes were identified to be involved in calcium, chemokine, cytokine-cytokine receptor interaction, MAPK, mTOR, neurotrophin, p53 and Toll-like receptor signaling pathways. The interplay between miRNAs and their mRNAs involving neuronal death and the above signaling pathways could be important in soman-induced brain injury development. This study is the first to investigate the role of miRNAs and their mRNA targets in the pathogenesis of brain injury after exposure to the chemical warfare agent soman

Disclosures: **J.M. Pizarro:** None. **T.L. Dao:** None. **J.A. Leuschner:** None. **S.W. Kaski:** None. **L.J. Shumway:** None. **C.R. Braue:** None. **R.K. Kan:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.01/Z12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NRF (Korea) 2013R1A2A1A03071089

Title: Effects of genetic liability to schizophrenia on intrinsic functional connectivity in the language network

Authors: ***S.-Y. KIM**[1], J.-W. HUR[2], Y. YOON[1], C. LEE[1], J. KWON[3]
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Abstract: Language dysfunction is one of the core features of schizophrenia (Li et al., 2009). Abnormal language processing in schizophrenia has also been demonstrated at the neural level, including aberrant neural connectivity between inferior frontal (Broca's area) and superior temporal (Wernicke's area) gyri in the patients. Using resting-state functional magnetic resonance imaging (rs-fMRI), the current study investigated effects of genetic liability to schizophrenia on intrinsic functional connectivity of the language network. Twenty nine patients with first-episode psychosis (FEP), 26 healthy individuals with genetic high risk to schizophrenia (GHR), and 51 age-matched healthy controls (HC) underwent rs-fMRI scans. For members of the GHR group, the Family Interview for Genetic Studies was used to investigate their family history of psychiatric disorders, and a quantitative measure of genetic liability was calculated for each participant to model likely exposure to genetic risk for schizophrenia (i.e., genetic liability

scale: GLS). Rs-fMRI scans were analyzed using CONN toolbox of SPM8 to determine intrinsic functional connections between brain regions. Broca's area (i.e., the pars opercularis and pars triangularis of the inferior frontal gyrus (IFG)) was utilized as a seed region to examine functional connectivity of the language network in each participant. The results showed strong functional connectivity between the seed area and language-related brain regions in HCs, including the bilateral IFG and the bilateral superior and middle temporal gyri (STG and MTG). Critically, we found significant hypo-connectivity between Broca's area and other language-related regions in both FEP and GHR groups compared to HCs. Specifically, patients showed significantly reduced connectivity between Broca's area and bilateral fronto-temporal areas. Compared to HCs, the GHR group showed significant hypo-connectivity between Broca's area and Wernicke's area in the left hemisphere. Importantly, a multiple regression analysis with the GLS as an independent factor in the GHR group revealed a significant effect of genetic liability to schizophrenia on functional connectivity between these two language areas: the greater the genetic liability in a GHR individual, the lesser the connectivity between Broca's area (BA44/45) and Wernicke's area (BA37) ($r=-.62$, $R^2=.38$, $p<.001$). In conclusion, the current findings suggest that aberrant functional connections in the left hemisphere language pathway may underlie an inherited vulnerability for schizophrenia, independent of medication or clinical symptom status.

Disclosures: S. Kim: None. **J. Hur:** None. **Y. Yoon:** None. **C. Lee:** None. **J. Kwon:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.02/Z13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Exploring neuropathological deficits and new drug targets for major psychiatric disorders using the Stanley Neuropathology Consortium datasets and RNA-Seq data

Authors: *S. KIM
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Abstract: The Stanley Neuropathology Consortium Integrative Database (SNCID, http://sncid.stanleyresearch.org) is a data-mining tool that includes 3586 neuropathological data sets as well as raw RNA-Seq data measured in two sample collections. The data can be used to further understand the aetiology of major psychiatric diseases and to potentially identify new

drug targets for these disorders. The two collections include the Neuropathology Consortium, which consists of 15 well-matched cases in each of four groups: schizophrenia (SCH), bipolar disorder (BPD), depression (MD) and unaffected controls (UC), and the Array Collection, which consists of 35 cases in each of three groups: SCH, BPD and UC. We reanalysed the neuropathological markers in multiple brain regions to identify those abnormalities that are shared between psychiatric disorders and those that are specific to each disorder. We then performed gene co-expression network analyses to identity the co-expression modules associated with each disease and also with abnormal markers in the hippocampus using the RNA-Seq data. Of the 2672 consortium collection data sets, 254 showed a significant abnormality in at least one disorder as compared to UCs. In the cortex, perineuronal oligodendrocytes and related markers were significantly altered in all three groups; SCH, BPD and UC whereas, the density of parvalbumin-containing neurons and related markers were significantly altered in just SCH and BPD, as compared to UCs. In the hippocampus, reelin-containing neurons and related markers were abnormal in the all three disease groups, whereas the density of parvalbumin-containing neurons and related markers were altered in SCH and BPD only. Co-expression modules that included immune and inflammation related genes were associated with SCH and BPD but not with MD. The immune/inflammation modules were also associated with the density of parvalbumin-containing neurons. The increased expression of immune and inflammation-related genes in the hippocampus may contribute to the deficits in the parvalbumin-containing neurons in SCH and BPD. The data further supports the possibility that drugs that impact the immune/inflammation system may be beneficial for the treatment of SCH and BPD.

Disclosures: S. Kim: None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.03/Z14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Grant from Lieber Institute

Title: Cis-acting regulation of a novel AS3MT transcript by a genome-wide supported risk variant for schizophrenia

Authors: *M. LI, R. TAO, A. E. JAFFE, F. ZHANG, C. LI, J. E. KLEINMAN, T. M. HYDE, J. SHIN, D. R. WEINBERGER
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Abstract: Background: Recent meta-analyses of genome-wide association studies for schizophrenia have reported strong associations of single nucleotide polymorphisms (SNPs) spanning Chromosome 10q24.32, in an about 757.5 kb genomic region covering numerous genes. However, little is known regarding the molecular mechanisms of the risk association. The top significant associated SNP rs7085104 is located about 500bp upstream of the classic translation start site of the arsenic (+3 oxidation state) methyltransferase (AS3MT) gene. This SNP has also shown associations with bipolar disorder (BPD) and major depressive disorder (MDD). **Methods:** Using RNA-Seq techniques (Hi Seq 2000, 80-120M 100 bp paired end reads) in the dorsolateral prefrontal cortex (DLPFC) of postmortem human brain tissue from normal controls (N=167), patients with schizophrenia (N=107), BPD (N=22) and MDD (N=96), and normal fetal brain (N=25) from a mixed race sample. **Results:** The SNP rs7085104 was significantly associated with AS3MT gene-level expression in healthy controls ($p<1.0\times10\text{-}4$). No other genes in the LD region showed significant expression associations with rs7085104. Furthermore, we identified novel junction reads within AS3MT, spanning from exons 1 to 4, but missing exons 2 and 3 (named junctiond2d3). The risk allele was associated with higher junctiond2d3 expression ($p<1.0\times10\text{-}3$), which is consistent with the diagnostic association of higher expression in patients ($p<1.0\times10\text{-}3$). In silico analysis predicted a truncated AS3MT transcript lacking exons 2 and 3 (denoted AS3MTd2d3), with a novel KOZAK sequence and translation start site in exon 4, which may encode a shortened protein lacking 102 amino acids. **Conclusions:** Our data suggest that the genetic risk variants in Chr.10q24.32 have potential cis-regulatory transcriptional effects on AS3MT,and more specifically, the truncated transcript AS3MTd2d3, which should be explored in future biological studies

Disclosures: M. Li: None. **R. Tao:** None. **A.E. Jaffe:** None. **F. Zhang:** None. **C. Li:** None. **J.E. Kleinman:** None. **T.M. Hyde:** None. **J. Shin:** None. **D.R. Weinberger:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: a grant-in aid for general scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan

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Title: The DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene are potent diagnostic biomarker in psychiatric disorders

Authors: *M. FUCHIKAMI[1], S. OKADA[1], S. YAMAWAKI[1], S. MORINOBU[2]
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Abstract: Major depression, because of its recurring and life-threatening nature, is one of the top 10 diseases for global disease burden. Major depression is still diagnosed on the basis of clinical symptoms in patients. The search for specific biological markers is of great importance to advance the method of diagnosis for depression. We examined the methylation profile of 2 CpG islands (I and IV) at the promoters of the brain-derived neurotrophic factor (BDNF) gene, which is well known to be involved in the pathophysiology of depression. We analyzed genomic DNA from peripheral blood of 20 Japanese patients with major depression or schizophrenia, and 18 healthy subjects to identify an appropriate epigenetic biomarker to aid in the establishment of an objective system for the diagnosis of depression. Methylation rates at each CpG unit was measured using a MassArray® system (SEQUENOM), and 2-dimensional hierarchical clustering analyses were undertaken to determine the validity of these methylation profiles as a diagnostic biomarker. Analyses of the dendrogram from methylation profiles of CpG I, but not IV, demonstrated that classification of healthy subjects and patients at the first branch completely matched the clinical diagnosis. At the next branch, classification of depression and schizophrenia by methylation profiles of CpG I, was also in complete accordance with the clinical diagnosis. Despite the small number of subjects, our results indicate that classification based on the DNA methylation profiles of CpG I of the BDNF gene may be a valuable diagnostic biomarker for psychiatric disorders.

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Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.05/Z16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The molecular and cellular neurobiology of psychotic disorders

Authors: *N. GUNHANLAR[1], F. M. S. DE VRIJ[1], G. SHPAK[1], C. G. BOUWKAMP[1], B. LENDEMEIJER[1], L.-A. GOUTY-COLOMER[1], M. GHAZVINI[2], T. M. W. Y. LI[2], V. BONIFATI[3], J. GRIBNAU[2], S. A. KUSHNER[1]
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Abstract: Psychotic disorders such as schizophrenia and bipolar disorder are quite common, affecting 2% of the general population. However, there is yet no satisfying explanation for the biological mechanisms underlying these disorders, due to the extraordinary complexity of the brain and the difficulties inherent in studying human neurophysiology. Applying Induced Pluripotent Stem (iPS) cell technology creates the unique opportunity to study neural cell cultures of individual patients. Our goal is to establish iPS-derived neural cell cultures from families with a high incidence of psychotic disorders and compare their intrinsic neuronal properties and network properties. To avoid heterogeneity caused by the wide range of manifestations of these diseases, we focus our efforts on families. Moreover, we have established a robust neural differentiation protocol that results in mature neurons and glial cells that together form a spontaneously active network confirmed by electrophysiology and confocal imaging. In parallel, high resolution exome sequencing is performed on DNA isolated from blood samples of patients and their healthy family members to find a genetic correlate of the disease in these families.

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Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Identifying targets of neuropsychiatric disease-associated transcription factors TCF4 and ZNF804A

Authors: *V. L. REINHART, S. XI, N. MATLUCK, C. SCHUBERT, T. A. LANZ
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Abstract: Large numbers of candidate genes and risk loci are emerging from recent GWAS and *de novo* exome sequencing studies for schizophrenia. Among them, several transcription factors have been strongly implicated by multiple lines of genetic evidences, including common variation, de novo mutations, rare variants, and copy number variation. However, the functions of these transcription factors are largely unknown. Identifying their direct targets may provide biological context and help uncover convergent pathways for these disease-associated genes, as well as facilitate the interpretation of differential gene expression observed in post-mortem brain samples from patients. The present study sought to uncover neuronal targets of two such transcription factors: transcription factor 4 (TCF4) and zinc finger protein 804A (ZNF804A). Myc-tagged constructs for each gene were over-expressed in HEK cells, and fixed lysates were analyzed by ChIP-sequencing against myc and RNApolII to identify the transcription factor binding motif and actively translating targets of the transcription factors. In parallel, viral shRNA constructs were developed against each gene and used to transduce rat primary cortical neurons at 7 DIV. After 14 DIV, total RNA was extracted and analyzed by RNA-sequencing. Genes overlapping in both the ChIP-sequencing and RNA-sequencing datasets were analyzed using Ingenuity Pathway Analysis, and select genes were validated in replicate neuronal experiments by qRT-PCR. TCF4 targets included several genes involved in actin dynamics (e.g. LIMK1, PAK4, MYLK), ERK signaling (e.g. MAPK3). Pathway analysis highlighted inflammatory cascades for both TCF4 and ZNF804A knockdown in primary neurons. Pathway analysis, gene targets and binding motifs for both transcript factors will be presented in full. Our data suggest that these GWAS-associated transcription factors impact pathways previously observed to be dysregulated in post-mortem brains from subjects with schizophrenia.

Disclosures: V.L. Reinhart: A. Employment/Salary (full or part-time):; Pfizer Inc. **S. Xi:** None. **N. Matluck:** None. **C. Schubert:** None. **T.A. Lanz:** None.

Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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KG Jebsen Foundation

Title: Evidence for the breakdown of neurotransmitter integration pathways in schizophrenia

Authors: A. DEVOR[1,5], W. K. THOMPSON[2], Y. WANG[3], P. SVENNINGSSON[6], A. J. SCHORK[4], V. ZUBER[7,8], C.-H. CHEN[2], S. DJUROVIC[7,8,9], R. S. DESIKAN[3], L. K. MCEVOY[3], O. A. ANDREASSEN[7,8], *A. M. DALE[1]
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Abstract: In polygenic traits and diseases, such as schizophrenia (SCZ), individual genetic loci account for only a very small portion of the phenotypic variance. Therefore, in addition to increasing the sample size, a key to improved yield from SCZ genome-wide association studies (GWAS) is the application of refined statistical methods, providing greater power for detection of small genetic effects, for a given sample size. To this end, we have recently developed a novel statistical methodology that improves power for gene discovery in GWAS by using a priori information about relative "enrichment" of statistical association based on genomic annotations (1). Application of this approach to summary statistics from the published Psychiatric Genomics Consortium SCZ GWAS indicated approximately three-fold increase in gene discovery over standard methods. The analysis revealed gene loci spanning multiple biological themes including numerous receptors for neurotransmitters and ion channels. Each gene has been discovered individually and independently of its function. The presence of hits across different neurotransmitter systems and channels for various ions begs the question of whether these hits reflect independent mechanisms, or are tied together through common biological pathways and

functions. To address this question, we have examined the identified gene loci for the presence of putative elements of previously described “neurotransmitter integration network” (2). Our results show that many of these genes indeed are mapped onto a common network, where the signaling cascades triggered by activation of GPCRs can modulate ion channels and ionotropic receptors. This may be achieved by activation of the intracellular kinases and phosphatases ensuring the appropriate level of excitability, and thus neuronal output, given the intensity and modality of the input. Taken together, the spectrum of molecular risk factors identified in our study supports the concept of SCZ as an “associative” disorder: a breakdown in the communication across different slow and fast neurotransmitter systems through intracellular signaling pathways. This emergent model may unify a number of currently competing hypotheses, e.g., Dopaminergic, Glutamatergic, Calcium and (a more general) Second Messenger Hypothesis and explain why effective antipsychotic drugs have a “rich pharmacology”, i.e. affinity for several receptors. 1.Schork AJ, et al. (2013). PLoS genetics 9(4):e1003449. 2.Greengard P (2001). Science 294(5544):1024-1030.

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Poster

424. Psychosis: Genetic Mechanisms

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Program#/Poster#: 424.08/Z19

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: No abnormal hexanucleotide repeat expansion of C9ORF72 in Japanese schizophrenia patients

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Abstract: Abnormal hexanucleotide repeat expansion of C9ORF72 is known to cause neurodegenerative disorders such as frontotemporal dementia. Additionally, patients with psychotic symptoms are more likely to have abnormal hexanucleotide repeat expansion than are patients without them. We investigated the hexanucleotide repeat sizes of C9ORF72 in 466 Japanese schizophrenia patients. We found no abnormal hexanucleotide repeat expansion. In

conclusion, C9ORF72 may not be responsible for schizophrenia susceptibility in the Japanese population.

Disclosures: **Y. Yoshino:** None. **Y. Mori:** None. **S. Ochi:** None. **S. Ueno:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.09/Z20

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Blood homocysteine and schizophrenia evaluated by a Mendelian randomization analysis

Authors: ***M. KINOSHITA**[1], S. NUMATA[1], A. TAJIMA[2], A. NISHI[1], I. IMOTO[2], T. OHMORI[1]
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Abstract: Objective: Observational studies have potential limitations, such as confounding, reverse causation, associative election bias, and attenuation by errors (Davey Smith and Ebrahim, 2005). Mendelian randomization, which used genetic variants as instrumental variables for exposures to overcome these problems, is a method for assessing causal relationship in epidemiological studies (Taylor et al., 2013). We recently demonstrated that increased blood homocysteine levels may be associated with an increased risk of developing schizophrenia in the Japanese population by conducting the Mendelian randomization approach (Nishi et al., 2014). However, it is still unclear that the association of blood homocysteine levels and a risk of schizophrenia in the world-wide population. In this study, we investigated the association between blood homocysteine levels and schizophrenia in the world-wide population by conducting Mendelian randomization based on the MTHFR C677T polymorphism (rs1801133). Methods: To estimate the association between rs1801133 and schizophrenia, we performed a meta-analysis of 33 case-control studies with the random-effect model by ‘metafor’, an R package. A total of 10,734 cases and 14,036 controls were included in this meta-analysis. Results: The pooled per-allele odds ratio (OR) for schizophrenia was 1.1 (95% CI = 1.04-1.17; p = 1.5 x 10-3, in the random-effects model) with significant heterogeneity (I2 = 48.1%; $p < 0.05$). The funnel plot analysis indicated no evidence of publication bias in this meta-analysis ($p > 0.05$). In the Mendelian randomization analysis, we found a significant effect of homocysteine on schizophrenia risk, representing an OR of 1.82 (95% CI = 1.26-2.66; p = 1.6 x 10-3) for

schizophrenia per 1-SD increase in homocysteine, when using a pooled estimate of per-allele standardized β coefficient (0.16) of the effect of rs1801133 on homocysteine from a recent meta-analysis of genome-wide association studies comprising 44,147 individuals. Conclusions: Our study indicated that elevated blood homocysteine levels may increase the risk of schizophrenia in the world-wide population. Further replication studies will be needed in larger samples using multiple genetic variants.

Disclosures: M. Kinoshita: None. **S. Numata:** None. **A. Tajima:** None. **A. Nishi:** None. **I. Imoto:** None. **T. Ohmori:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH076060

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Title: Messenger and microRNA expression profiling in neurons and oligodendrocytes in schizophrenia and Parkinson's disease

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Abstract: The human brain is an extraordinarily complex structure consisting of heterogeneous subsets of neurons that mediate distinct aspects of information processing. Disturbances of these neurons compromise the functional integrity of the connectional architecture of the brain, resulting in various psychiatric and neurological disorders. To explore how the molecular integrity of various neural subtypes might be compromised in psychiatric and neurodegenerative diseases, we determined the mRNA and miRNA expression profiles of pyramidal, parvalbumin (PV)-immunoreactive, and dopamine neurons, and oligodendrocytes in schizophrenia (SZ) or Parkinson's disease (PD), and ascertained the convergence and specificity of the transcriptional networks and signaling cascades that are altered in these disorders In pyramidal neurons from the

superior temporal cortex in SZ, we found differentially expressed mRNAs that belong to the transforming growth factor beta and the bone morphogenetic proteins signaling pathways. In the PV neurons from the same region, differentially expressed transcripts were associated with WNT, NOTCH, and PGE2 signaling and transcription factors such as LHX6, in addition to genes that regulate cell cycle and apoptosis. In PD dopamine neurons of the substantia nigra, there was a predominant down-regulation of mRNAs, including PARK gene family members and genes associated with programmed cell death, mitochondrial dysfunction, neurotransmitter and ion channel receptors, as well as an upregulation of transcripts that are involved in neuronal survival mechanisms. We also assessed oligodendrocytes from SZ subjects, which exhibited a distinct expression pattern that is consistent with dysregulation of cell cycle events. In addition to the mRNA expression profiles, we identified a set of differentially expressed miRNAs in both SZ and PD. Enrichment analysis of their predicted targets or from negative correlation analyses, revealed an association of miRNAs with dysregulated signaling pathways, raising an interesting possibility that dysfunction of neurons or oligodendrocytes in SZ and PD may in part be mediated by a concerted dysregulation of gene network functions as a result of the altered expression of miRNAs. Our data show mostly distinct, but also overlapping dysfunctional gene and miRNA networks between different neural cell populations in SZ and late stage PD, and provide a platform for future downstream analyses aiming to understand the disease-specific and shared molecular processes of individual neuronal dysfunction in these disorders.

Disclosures: S.A. Mauney: None. **K.C. Sonntag:** None. **T.W. Woo:** None.

Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH076060

MH080272

Title: Clusterin immunoreactivity in the cerebral cortex in subjects with schizophrenia

Authors: *K. M. ATHANAS[1], S. DASDELEN[1], T.-U. W. WOO[1,2,3]
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Beth Israel Deaconess Med. Ctr., Boston, MA; [3]Dept. of Psychiatry, Harvard Med. Sch., Boston, MA

Abstract: Clusterin is a multifunctional disulfide-linked heterodimeric chaperone protein that is involved in many biological processes. Several studies have found clusterin dysregulation to contribute to a number of disorders, from tumorigenesis to neurodegenerative states, such as Alzheimer's disease, by acting to either promote or inhibit oxidative stress, apoptosis, synaptic plasticity, amongst other functions. Because of our recent observations that the mRNA that encodes clusterin was upregulated by more than 2-fold in both pyramidal and parvalbumin-containing inhibitory neurons in the cerebral cortex in schizophrenia, in this study, we address the hypothesis that clusterin protein expression will also be increased in subjects with schizophrenia. In a cohort of postmortem brains from 20 schizophrenic and 20 demographically matched normal control subjects obtained from the Harvard Brain Tissue Resource Center, we immunohistochemically visualize the cellular localization of clusterin in the dorsolateral prefrontal cortex (Brodmann's area 9) and primary visual cortex (Brodmann's area 17). Qualitative examination reveals that clusterin is expressed in various cell types, including both pyramidal and non-pyramidal neurons in addition to glial cells across all cortical layers. Quantification of the densities of clusterin-immunoreactive cell subtypes is underway. Findings of this study will determine if clusterin expression is disturbed in schizophrenia and will thereby shed light on the possible mechanistic link between this protein and known pathophysiological processes of the illness, such as oxidative stress, cellular injury and synaptic deficit.

Disclosures: K.M. Athanas: None. **S. Dasdelen:** None. **T.W. Woo:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH T32 5T32MH015330-36

Title: Serine Racemase (SRR) and schizophrenia risk: Functional genomic characterization of schizophrenia GWAS risk variants

Authors: *R. BIRNBAUM[1], F. ZHANG[2], T. M. HYDE[2,3], J. E. KLEINMAN[2], D. R. WEINBERGER[1,4]

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Abstract: Background: Converging evidence supports the hypothesis that N-methyl-D-aspartate(NMDA)-type glutamate receptor hypofunction is pathogenic in schizophrenia. D-serine, a co-agonist required for full activation of the NMDA receptor, is synthesized by serine racemase. Recently SRR rs423957 was reported to be associated with schizophrenia in a PGC meta-analysis (p-value =5.69x10-8, OR=1.1) (Ripke et al Nat Gen 2013). The current analysis investigated the main effect of the risk associated SNP and correlated SNPs on SRR abundance by RNA Sequencing methodology. We also investigated the effect of the risk associated SNP and correlated SNPs on antipsychotic drug response. **Methods**: RNA sequencing was performed on post-mortem PFC grey matter for 101 Caucasian controls (27 females, 38 +/- 23 years, 74 males, 36 +/-18 years) and 64 SCZ cases (13 females, 53+/- 18 years, 51 males, 42 +/- 13 years). Samples were enriched with PCR to create a cDNA library for high throughput sequencing. Pair-end reads of cDNA sequences were aligned the human genome reference (TopHat v2.0.4). Genotype effect on time to discontinuation of medication, the primary outcome measure in the CATIE study, was determined using the Cox proportional hazards ratio model, while controlling for age, sex, duration of illness, and drug clearance. **Results**: SRR DLPFC expression was reduced in schizophrenia cases compared to controls ($F(1,117)=1.2$, $p=0.035$). The risk SNP rs423957 was associated with decreased SRR abundance in CAUC controls (p-value=0.03) and in CAUC schizophrenia samples (p-value=0.0023), and SNPs in LD with rs423957 showed highly significant association with SRR abundance. For an alternative proxy SNP in LD ($r2=0.89$) with the GWAS SNP, there was a hazard ratio of 2 for the minor allele in a pharmacogenetic analysis of time to discontinuation of antipsychotic medication. **Conclusions**: The current results of decreased SRR mRNA associated schizophrenia confirm previous reports of decreased D-serine associated with schizophrenia. The cis-eQTL effect of the PGC risk SNP and of variants in LD with it, suggest a putative mechanism of risk. Risk variant association with other intermediate phenotypes, including neuroimaging and cognition phenotypes, pertinent to schizophrenia risk, is ongoing.

Disclosures: R. Birnbaum: None. **F. Zhang:** None. **T.M. Hyde:** None. **J.E. Kleinman:** None. **D.R. Weinberger:** None.

Poster

424. Psychosis: Genetic Mechanisms

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Program#/Poster#: 424.13/Z24

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Maternal infection in mice leads different DNA methylation and gene expression between male and female offspring

Authors: *Z. YU[1], R. FUNAYAMA[2], K. UENO[3], N. NARIAI[3], K. KOJIMA[3], C. ONO[1], Y. KASAHARA[1], Y. KIKUCHI[1], M. NAGASAKI[3], K. NAKAYAMA[2], H. TOMITA[1,4]
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Abstract: Background: Evidence suggests that both genetic and environmental factors are associated with schizophrenia. Environmental components, such as maternal immune responses to infection during pregnancy have been considered a risk factor for schizophrenia. Previous mouse studies showed that maternal immune challenge induced schizophrenia-related abnormal behavior in adults offspring. Additionally, postmortem studies have reported the alterations in gene expression and DNA methylation in schizophrenia. Taken together, maternal infection may influence genetic expression and DNA methylation during postnatal brain development, which involve in the pathogeneses of schizophrenia. Purpose: To investigate the transcriptional regulations affected by maternal immune challenge in fetal brain by microarray and ChIP-seq, which remains throughout development and cause behavioral abnormality in adult. Methods: Poly I:C or PBS was administered to pregnant mice (C57BL/6) every 6 consecutive days from E14 to E19. The offspring were separated from their mothers after 3 weeks and divided into 4 groups: `Poly I:C-female`, `Poly I:C-male`, `PBS-female` and `PBS-male` -mice groups. After behavioral evaluations, total RNA from prefrontal cortex of adults offspring were applied on Illumina Mouse WG-6 V2 Beadchips to analyze mRNA expression profiling. Methylated DNA was isolated from prefrontal cortex using MethylMiner Methylated DNA Enrichment Kit. Illumina Genome Analyzer IIx was used to analyze DNA methylation profiling. Sequenced reads were mapped to the mouse genome (UCSC mm9), and target genes-associated peak calls were determined with MACS and GREAT software. Results and conclusion: Gender difference was observed in PPI study; Poly I:C-male mice showed decreased %PPI only in 71 dB ($P<0.05$), whereas Poly I:C-female mice showed significantly decreased %PPI in all of 68 dB ($P<0.01$), 71 dB ($P<0.001$) and 77 dB ($P<0.001$). Specific gene expression profiles and methylation patterns were identified in both of Poly I:C-male and -female mice comparing with its PBS-control, respectively. Poly I:C-female mice showed more genes transcript alterations and DNA hypermethylation than male counterparts. Data suggest molecular mechanisms underlying the impact of maternal immune response to the fetal brain, which is preserved until adult and causes behavioral abnormality in gender-specific manner.

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Poster

424. Psychosis: Genetic Mechanisms

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Program#/Poster#: 424.14/Z25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: No evidence of association of schizophrenia-risk genotype in rs1625579 with expression of mir137 in postmortem human prefrontal cortex

Authors: N. FENG, V. IMAMOVIC, *B. K. LIPSKA
NIH/NIMH, BETHESDA, MD

Abstract: A large genome-wide association study identified a locus on chr 1p21.3, rs1625579, located in the intronic region of MIR137 host gene, as strongly associated with schizophrenia, and later with autism spectrum disorder. In subsequent studies, a risk-associated common allele (T) was shown to predict the severity of cognitive deficits in schizophrenia, regional brain activity, and changes in brain structure. We sought to determine the effects of rs1625579 on molecular phenotype by analyzing expression of MIR137 mRNA in the dorsolateral prefrontal cortex (DLPFC) of 286 subjects (198 controls and 88 patients with schizophrenia), ranging in age from birth through old age (over 80 years old). We also examined separately the effect of diagnosis in 231 adult subjects (over 18 years old, 143 controls and 88 subjects with schizophrenia). RNA was extracted using miRNeasy Kit (QIAGEN). Reverse transcription was conducted using specific miRNA primers (ABI). TaqMan qPCR assay measured MIR137 expression levels that were normalized to a geometric mean of U6 and U44 small nucleolar RNA (snoRNA). The genotypes at rs1625579 were obtained using Illumina BeadArrays (1M). We used analysis of covariance (ANCOVA) with genotype and diagnosis as categorical variables, and age, sex, pH, postmortem interval (PMI) and RNA integrity (RIN) as covariates. We also conducted ANCOVA with race (157 African Americans and 71 Caucasians) and genotype as independent variables. Expression of MIR137 mRNA slightly decreased with age throughout the lifespan in all subjects ($r=-0.35$, $p=0.0000$), and in controls and schizophrenia patients when analyzed separately ($r=-0.36$, $p=0.0000$, and $r=-0.22$, $p=0.03$, respectively). There was no significant effect of genotype at rs1625579 in all subjects ($F=0.3$, $p=0.7$) and when in controls

were analyzed separately (F=1.5, p=0.2). There was also no significant effect of diagnosis (F=0.08, p=0.8) on expression of MIR137 mRNA, and no significant genotype by diagnosis interaction. Moreover, we did not detect a significant effect of race, and there was no race by genotype interaction on MIR137 mRNA expression (F=0.3, p=0.8). In conclusion, we did not find a difference in MIR137 expression between controls and patients with schizophrenia or a significant effect of genotype at rs1625579 on MIR137 mRNA expression in the DLPFC. The molecular mechanisms underlying clinical association with schizophrenia remain elusive.

Disclosures: **N. Feng:** None. **V. Imamovic:** None. **B.K. Lipska:** None.

Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant UL1-DE019580

NIH Grant RL1MH083269

Title: Common variants for schizophrenia ascertained through genome-wide association with a cognitive endophenotype

Authors: ***A. B. ZHEUTLIN**[1], R. G. FORTGANG[1], K. M. HAUT[1], F. W. SABB[2], R. M. BILDER[3], N. FREIMER[4], E. D. LONDON[4], T. D. CANNON[1]
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Abstract: Background: Schizophrenia is highly heritable, but also genetically complex and heterogeneous, likely involving 1000s of common variants. Common variant effect sizes for any complex measure (e.g., schizophrenia, intelligence) are often too small to pass genome-wide significance, even employing extremely large samples. One approach to overcoming this lack of power is to create a polygenic risk score combining risk across SNPs (including those not meeting genome-wide thresholds for significance) and weighted individually by their effect sizes. Here, we directly tested whether polygenic variants associated with an endophenotype for schizophrenia - verbal memory performance - in healthy controls also predict memory performance in patients with schizophrenia. We also explicitly tested the implications of the

common variant, common disease model by examining the relationship between endophenotype-generated scores and schizophrenia-related risk scores in the same sample. **Methods:** Healthy controls ($N = 900$) and schizophrenia patients ($N = 58$) completed the California Verbal Learning Task, a list-learning measure of verbal memory. A bootstrapping analysis of associations between CVLT performance and common variants was run (100 iterations) in the control sample and multiple cut-points (SNPs present in 70, 80, and 90 of 100 associations) were used to calculate CVLT polygenic scores. Schizophrenia risk scores were calculated using variants identified by the Psychiatric Genomics Consortium in a large, case-control sample. **Results:** For two of the three replication/resampling cut-points, scores derived from weights based on the control sample revealed significant associations with CVLT performance in patients with schizophrenia. Lower schizophrenia risk scores were associated with higher CVLT polygenic scores (CVLT scores and performance are positively correlated) at all three replication/resampling cut-points in patients with schizophrenia. **Conclusions:** Polygenic memory scores reflecting variation in CVLT performance in healthy controls significantly predicted CVLT performance in a small sample of schizophrenia patients. This is the first demonstration, to our knowledge, of polygenic scores derived from an endophenotype-based, genome-wide association study in schizophrenia successfully predicting the same phenotype (i.e., memory performance) in an independent sample. Furthermore, the relationship between schizophrenia risk scores and CVLT-derived scores provides the first genome-wide, molecular genetic evidence of the shared etiology between episodic memory performance and schizophrenia.

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Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD Grant 21003

Title: Exploring the genome in three dimensions in the prefrontal cortex: Discovering the functional relevance of non-coding regions of the genome

Authors: *A. C. MITCHELL[1], P. ROUSSOS[1], V. POTHULA[1], A. LESSARD[2], S. AKBARIAN[1]
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Abstract: Less than 1.5% of the human genome encodes protein. However, vast portions of the human genome are transcriptionally and epigenetically regulated. Many noncoding regulatory DNA elements are thought to regulate the spatial organization of interphase chromosomes. Chromosomal 'loopings', for example, are pivotal for the orderly process of gene expression. They enable distal regulatory enhancer or silencer elements to interact directly with proximal promoter and transcription start sites potentially bypassing many kilobases of interspersed linear genomic sequence. To date very little is known about the regulation of these supranucleosomal structures in brain nuclei. Methods: We introduce chromosome conformation capture (3C) for brain and compare higher-order chromatin structures at schizophrenia risk loci. Results: We show that chromosome conformation capture, a widely used approach to study higher-order chromatin, is applicable to tissue collected postmortem, thereby informing about genome organization in the human brain. We highlight loops positioned at H3K4me3 marks at HLA-DRB9, HIST1H2BJ, and CACNA1C. Furthermore, we introduce circular chromosome conformation capture (4C) to detect looping interactions between specific loci and across the genome. Conclusions: We provide evidence that chromosomal loopings exist between schizophrenia risk loci at H3K4me3 marks between (1) genes, (2) intergenic SNPs and transcription start sites, and (3) transcription start sites and intronic SNPs in postmortem prefrontal cortex. Chromosomal loopings are often accompanied with the presence of histone marks. H3K4me3 ChIPseq data shows that these marks are altered at intergenic and intronic loci in schizophrenia, suggesting that intergenic chromosomal loopings may be altered in schizophrenia. We predict that the exploration of three-dimensional genome architectures and function will open up new frontiers in human brain research and psychiatric genetics and provide novel insights into the epigenetic risk architectures of regulatory noncoding DNA. This research is funded by the Brain and Behavior Research Foundation and the National Institutes of Mental Health.

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Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Association of a GWAS schizophrenia risk DRD2 locus (an established antipsychotic target) with inefficient DLPFC activation during fMRI in healthy controls and in schizophrenia

Authors: *E. RADULESCU[1], Q. CHEN[1], J. H. CALLICOTT[2], V. S. MATTAY[1], D. R. WEINBERGER[1]
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Abstract: Background: The D2 receptor gene (DRD2) is the only gene so far that encodes for a known antipsychotic drug target. Recent Genome-Wide Association Study (GWAS) results based on the Psychiatric GWAS Consortium (PGC) data found an association at the DRD2 locus with risk for schizophrenia. While the D2 receptor protein is an established drug target, we tested the association of the DRD2 locus (using a significant single nucleotide polymorphism- SNP, rs2514218) with a previously validated intermediate phenotype of schizophrenia [1]-right dorso-lateral prefrontal cortex (DLPFC) activation during a working memory fMRI paradigm. Methods: 128 healthy volunteers and 58 individuals with schizophrenia (DSM-IV-TR), genotyped for DRD2 rs6589377, a proxy ($r2>0.8$) for rs2514218 (the SNP significant in the GWAS data), participated in an fMRI working memory study (Nback). The subjects were matched for performance during the 2-back condition (accuracy $\geq$ 70%). Individual first-level GLM contrast images (2-back > 0-back) were analyzed within the SPM8 ANCOVA (genotype, diagnosis = between-subjects factors; age, IQ, performance, reaction time: covariates). Results were corrected for multiple comparisons at the whole-brain level and within pre-defined DLPFC regions. Results: We found a statistically significant association of rs6589377 with prefrontal engagement: the highest activation in the right DLPFC (consistent with a phenotype of inefficiency) was found for GG (homozygotes for minor-risk allele) > AG > AA (homozygotes for major allele) (cluster peak at MNI: 30 42 39, R-BA9, Z score=4.40, $p<0.001$ FWE corrected for multiple comparisons). This effect was even more pronounced in the schizophrenia individuals. No genotype x diagnosis interaction was observed. Conclusions: Our findings support the functionality of the significant DRD2 locus variation at a brain system level. Our results also support the idea that intermediate biological phenotypes associated with risk for schizophrenia may be valid biomarkers for evaluating potential antipsychotic effects of novel therapeutic agents. [1] Rasetti R, Weinberger DR. Intermediate phenotypes in psychiatric disorders. Curr Opin Genet Dev. 2011 Jun;21(3):340-8.

Disclosures: E. Radulescu: None. **Q. Chen:** None. **J.H. Callicott:** None. **V.S. Mattay:** None. **D.R. Weinberger:** None.

Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH074791

Title: PDE10A isoform heterogeneity and expression within the human striatum

Authors: C. MACMULLEN, K. VICK, R. PACIFICO, *R. L. DAVIS, Prof
Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: Bipolar Disorder (BD) is a neuropsychiatric disorder characterized by severe mood swings that affects nearly 5.7 million American adults according to the National Institute of Mental Health. Genetic studies have associated the cAMP signaling pathway phosphodiesterase PDE10A to this disorder. PDE10A inhibitors are also under intense study as novel therapeutics for neuropsychiatric disorders. Two annotated isoforms of PDE10A, PDE10A2 and PDE10A1, differ only in alternately spliced 5' exons. These exons are separated by a large intron known to possess twelve common SNPs and eight rare SNVs associated with BD. To learn more about the genetic organization of this gene region, 5' RNA Ligase Mediated Rapid Amplification of cDNA Ends (RLM-RACE) as well as high throughput RNA sequencing studies of human striatal brain tissue were employed to uncover novel splice isoforms of PDE10A. Anti-peptide antibodies were generated to these PDE10A isoforms and their cellular distribution within mouse cortical and striatal neurons was explored using immunocytochemistry. The interactions of these isoforms were also analyzed by over-expressing the proteins in HEK293 cells. Finally, isoform specific antibodies were used in immunoprecipitation assays to illustrate the presence of these proteins within non-psychiatric disease control striatal tissue. These studies extend our current knowledge of PDE10A isoform diversity within the human striatum. They also highlight novel targets to pursue for developing anti-psychotic drug therapies.

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Poster

424. Psychosis: Genetic Mechanisms

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Polygenic risk scores for schizophrenia predict hippocampal function

Authors: *Q. CHEN, V. S. MATTAY, E. Y. XIAO, R. E. STRAUB, D. R. WEINBERGER
Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: Introduction: In recent years, polygenic risk profile scores (RPS) are being increasingly used to indirectly measure the collective genetic effect of many weakly associated markers on the risk for a neuropsychiatric disorder and for their modulatory role on neural circuits. Modeling RPS can help to investigate the polygenic component in a trait involving markers with small effects across the whole genome or certain gene-set genetic components (ISC 2009). In the current study we used RPS to study whole genome effect on a previously validated intermediate phenotype of schizophrenia, namely altered hippocampal engagement during a simple declarative memory task. Methods: Two hundred and five healthy volunteers underwent BOLD fMRI (3T) during a simple declarative memory task (SDMT), which included incidental encoding and retrieval of visual scenes. For both the encoding and retrieval sessions, the scenes were presented in a blocked fashion, with 4 blocks of neutral scenes and 4 blocks of visual scenes alternating with 9 blocks of resting state (fixation cross hair). In the current study, we focused on the encoding phase for neutral scenes only (Rasetti & Mattay et al. 2014). RPSs were calculated for each individual as the weighted sum of the number of reference alleles on preselected markers based on PGC GWAS study (ISC 2009). Scoring thresholds include 5E-8, 0.0000001, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5, and 1, and association testing was conducted in SPM8. Results: We found a significant inverse association between RPS and hippocampal activation for scoring thresholds of 0.01 and above. Association p values ranged from p=0.001 to p=0.003 (uncorrected). At the threshold of 0.01, RPS (24597 markers were included) accounted for 4.83% of the variance in right hippocampal activation. Conclusions: Our results show that polygenic risk profile scores for schizophrenia significantly correlated with altered hippocampal function. The relationship of higher RPS with decreased hippocampal function is consistent with our previous finding of decreased hippocampal engagement during a simple declarative memory task in patients with schizophrenia and their siblings when compared to healthy volunteers (Rasetti & Mattay et al.2014). This finding implies that a polygenic risk score approach can be used to explore the influence of the cumulative effect of risk genes on brain function, in particular on how risk genes collectively modulate neural circuits and confer risk for schizophrenia.

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Poster

424. Psychosis: Genetic Mechanisms

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Title: Functional characterization of L-type Calcium channel gene CACNA1C variants implicated in mood disorder genome wide association studies

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Abstract: The CACNA1C gene encodes the alpha-pore forming subunit of the Ca2+ channel CaV 1.2 (L-type voltage gated). Multiple genome wide association studies (GWAS) have indicated significant association between single nucleotide polymorphisms in CACNA1C and mood disorders, as well as schizophrenia. All such SNPs are located in intron 3 and therefore do not alter protein structure. Their biological implications and role in altering disease susceptibility remain unknown. The goal of this study was to analyze functionality of these genetic changes. We hypothesized that these associated SNPs, or other variants in linkage disequilibrium (LD) with them harbor regulatory elements that affect CACNA1C expression. We bioinformatically identified evolutionarily conserved regions within Intron 3 of CACNA1C, one of which contains 2 SNPs in LD with a GWAS significant SNP and putative binding sites for multiple transcription factors. Because this particular conserved region might play a regulatory role in CACNA1C expression, we cloned this intronic sequence, containing major and minor alleles of the 2 SNPs, into reporter gene vector and assessed their ability to for transcription activation in HEK293 cells and neuronal cell line SH-SY5Y. Our data showed that haplotype consisting of the minor alleles of both SNPs lead to significantly higher expression of the reporter gene. These data indicate that the putative regulatory region affects reporter activation in an allele specific manner. The data provides basis for focused study of intron 3 of the CACNA1C where all the CACNA1C mood disorder GWAS signals reside. We expect that further functional characterization of CACNA1C regulatory elements will lead to an improved understanding of the molecular mechanisms underlying pathophysiology of psychiatric disorders as modulated by CACNA1C genotype

Disclosures: **S.S. Bhat:** None. **R.J. Smith:** None. **M. Prasad:** None. **Y. Chang:** None. **T.D. Gould:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 424.21/Z32

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: SAF2010-20840-C02-01

SAF2010-20840-C02-02

Becas Lopez-Albo 2013- IDIVAL

Title: Altered expression of genes involved in immune and inflammatory response in drug-naïve first episode schizophrenia

Authors: ***B. CRESPO-FACORRO**[1,2], J. SAINZ[3], I. MATA[4], R. PEREZ-IGLESIAS[5], I. VARELA[6], M. ARRANZ[7], P. SUAREZ-PINILLA[4]
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Abstract: Schizophrenia is a mental disorder with severe consequences that has a lifetime risk of about 1% and heritability estimated at up to 80%. In order to characterize gene candidates and to obtain functional information regarding the molecular bases of the disease, mRNA from blood was sequenced using an Illumina Genome Analyzer GAIIx. 200 genes with significant differential expression after adjusting for multiple testing (Padj Value <0.05) were found in the 22.278 human genes analyzed. 37 genes out of the 200 differential expression genes (19%) have been already included in the GWAS catalog2, a slight enrichment regarding the 17% fraction of GWAS associated genes. Among these 200 genes, ADAMTS2 has the lowest Padj value

(1.3236E-69) and it has been related with attention deficit hyperactivity disorder (time of onset).3 Three other differentially expressed genes have been associated to schizophrenia in the GWAS: CSMD1 associated with schizophrenia among other traits4 and EHF associated with response to antipsychotic treatment and volumetric brain 5,6. RFX2 is not reported but includes an intronic SNP with a P value = 3.5x10-6 which ranks in position 89 among the P values of a schizophrenia and bipolar disorder GWAS meta-analysis.7 According to GeneRIF repository , 9 out of the 200 differentially expressed genes have been related to schizophrenia: GRIK3, LPL, S100B, SLC12A1, SNCA, SYN2, TUBB2A, SELENBP1, CSMD1. Levels of S100B have been reported to be increased in schizophrenia which is consistent with our observation of significantly higher expression of the gene in schizophrenics. Also, lower copy number of SELENBP1 in schizophrenics has been reported, which is consistent with the significant lower expression levels we observe in schizophrenics. Functional analyses of the differentially expressed genes using the tool FatiGO from the package Babelomics indicated a significant enrichment in 9 Gene Ontology biological processes (Padj Values <0.05). These processes are mainly related to protein processing and inflammatory and immune response. There are 20 differential expression genes involved in the immune, inflammatory response and the response to wounding, 15 of these genes are upregulated and 5 downregulated in schizophrenia.

Table 1. Significantly enriched gene Ontology biological processes

Biological Process	Differential Expression genes	Observed % of all	Expected % of all	Differential Expression symbols	Padj Value
Protein processing (GO:0016485)	8	4.00	0.53	ADAMTS2,C4BPA,CFD,KRT1,F12,VSIG4,CARD17,SERPING1	1.26E-02
protein maturation (GO:0051604)	8	4.00	0.53	ADAMTS2,C4BPA,CFD,KRT1,F12,VSIG4,CARD17,SERPING1	1.39E 02

protein maturation by peptide bond cleavage (GO:0051605)	7	3.50	0.42	ADAMTS2,C4BPA,CFD,KRT1,F12,VSIG4,SERPING1	1.39E 02
acute inflammatory response (GO:0002526)	9	4.50	0.81	C4BPA,CFD,KRT1,CEBPB,F12,VSIG4,SERPING1,FN1,VNN1	1.84E 02
activation of plasma proteins involved in acute inflammatory response (GO:0002541	6	3.00	0.24	C4BPA,CFD,KRT1,F12,VSIG4,SERPING1	1.26E 02
response to wounding	18	9.00	3.24	C4BPA,CFD,ENTPD2,GP1BB,SIGLEC1,TPST1,KRT1,NOTCH3,EREG,CEBPB,F12,VSIG4,SERPING1,SDC1,FN1,VNN1,MIF,FOXQ1	3.56E 02

(GO:0009611)					
innate immune response (GO:0045087)	10	5.00	0.80	C4BPA,CFD,KRT1,EREG,F12,VSIG4,SERPING1,VNN1,MIF,SNCA	1.26E 02

Disclosures: **B. Crespo-Facorro:** None. **J. Sainz:** None. **I. Mata:** None. **R. Perez-Iglesias:** None. **I. Varela:** None. **M. Arranz:** None. **P. suarez-pinilla:** None.

Poster

424. Psychosis: Genetic Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH MH077175

The Brain and Behavior Research Foundation (NARSAD)

Takeda Pharmaceuticals

the William P. and Henry B. Test Endowment

Title: Epigenetic factors can dysregulate the GABA cell phenotype in schizophrenia

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Abstract: GABAergic dysfunction in schizophrenia (SZ) is associated with a marked decrease in the expression of GAD67 in interneurons found in the stratum oriens (SO) of sector CA3/2 of the hippocampus (HIPP). A microarray-based gene expression analysis of laser microdissected (LMD) human hippocampus has suggested that GABA neurons in the SO of CA3/2 may contain a network of differentially regulated genes that are involved in GAD67 regulation. Several of these genes may contribute to the highly significant decrease of GABAergic activity at the SO-CA3/2 locus in SZ. To validate the role of this network in GAD67 regulation, both *in vitro* and *in vivo* studies in which shRNAi and lentiviral vectors have been used to explore how changes in the expression of GAD67 may be influenced by two other genes, HDAC1 and DAXX, that have been specifically implicated in the proposed network could potentially influence the expression of GAD67. Toward this end, knockdowns of HDAC1 and DAXX expression were induced both *in vitro* (in HiB5 cells with a GABAergic phenotype) and *in vivo* (in GABA cells of the SO of CA3/2 following selective stereotaxic infusions of the vectors). Suppression of HDAC1 and DAXX expression was associated with a highly significant increase of GAD67 expression; however, neither gene was associated with changes in the expression of its respective co-repressor (i.e. DAXX or HDAC1, respectively). Viral vector knockdowns of both HDAC1 and DAXX did, however, induce significant changes in the expression of other putative GAD67 regulatory genes, such as Runx2 and PAX5. Taken together, these findings suggest that both HDAC1 and DAXX are components of a complex network of genes identified in GABAergic neurons at a key locus of the trisynaptic pathway where significant abnormalities have been observed in patients with SZ. HDAC1 and DAXX are known co-repressors of methylation reactions at CpG islands and may be contributing to transcriptional regulation of GAD67 expression. In summary, these experiments have provided validatory information suggesting that there is a complex network of interactive genes that play a complex role in both normal and abnormal GABA cell function in the hippocampus.

Disclosures: S. Subburaju: None. **A.J. Coleman:** None. **F.M. Benes:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.01/Z34

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: A serotonin-dependent mechanism is essential for the protracted antidepressant-like effect of ketamine in a rat model of depression

Authors: *K. G. DU JARDIN[1], N. LIEBENBERG[1], H. MÜLLER[1], B. ELFVING[1], C. SANCHEZ[2], G. WEGENER[1]
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Abstract: Ketamine, a non-competitive NMDA receptor antagonist, produces an antidepressant effect within two hours that persists for more than one week in treatment-resistant patients. Likewise, ketamine exhibits a rapid and protracted antidepressant-like effect in a genetic model of depression, Flinders Sensitive Line (FSL) rats. The mechanisms mediating the antidepressant effect of ketamine are not well understood, however, the FSL rat model may offer opportunities to gain new mechanistic insights. Since cognitive deficits are common in depression, and FSL rats display reduced memory performance compared to their control strain, Flinders Resistant Line (FRL), FSL rats may constitute a valid platform to study cognitive aspects of depression. Moreover, numerous studies support that serotonin (5-HT) is implicated in antidepressant responsiveness and regulation of memory function. Thus, the objective was to assess the impact of endogenous 5-HT tone on ketamine's protracted antidepressant-like effect and any protracted effect on memory function by comparing its effect in rats with normal 5-HT tone to 5-HT depleted rats. FSL rats were pretreated with the irreversible tryptophan hydroxylase inhibitor, p-chlorophenylalanine (pCPA; *IP* 150 mg/kg/day), or saline once daily for three consecutive days. On the following day, the FSL rats were acutely treated with ketamine (*IP* 15 mg/kg) or saline. Saline/saline-dosed FRL rats were included as controls. Ketamine's effects on recognition memory and its antidepressant-like activity were assessed in the object recognition test (24 hours after ketamine) and the forced swim test (48 hours after ketamine), respectively. In the object recognition test, control FSL rats exhibited a significantly lower memory performance than control FRL rats. Neither, pretreatment with pCPA nor treatment with ketamine affected the memory performance of the FSL rats. In the forced swim test, control FSL rats displayed a significantly higher immobility than control FRL rats. Saline pretreatment combined with ketamine treatment significantly reduced the immobility of the FSL rats, whereas pCPA pretreatment alone did not affect immobility. However, pCPA pretreatment significantly prevented the antidepressant-like effect of ketamine treatment. In conclusion, 5-HT depletion prevented the protracted antidepressant-like effect of ketamine without affecting the depressive-like phenotype of FSL rats. These results suggest that ketamine elicits its antidepressant-like effect via a 5-HT-dependent mechanism. Neither ketamine nor 5-HT depletion affected the memory performance of FSL rats. Additional behavioral data will be presented.

Disclosures: K.G. Du Jardin: None. **N. Liebenberg:** None. **H. Müller:** None. **B. Elfving:** None. **C. Sanchez:** A. Employment/Salary (full or part-time):; Lundbeck Research USA, Inc.. **G. Wegener:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.02/Z35

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Time-dependent metabolomic profiling of Ketamine drug action reveals hippocampal pathway alterations

Authors: K. WECKMANN, C. WEBHOFER, C. LABERMAIER, M. MÜLLER-SITZ, *M. KIMURA, C. W. TURCK
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Abstract: Psychiatric diseases including Major Depressive Disorder (MDD) have a high morbidity and constitute an ever increasing burden for societies. At present most clinically used antidepressants are targeting monoaminergic reuptake mechanisms. However, a limited efficacy and a delayed onset of therapeutic response combined with several side effects make them less than ideal drugs. Approximately one third of patients are suffering from treatment resistant depression (TRD) and do not respond to commonly used antidepressants. Reasons for the delayed therapeutic effect and TRD remain elusive. To improve antidepressant drug efficacy, one line of research has focused on the *N*-methyl-*D*-aspartate receptor (NMDAR) and its signaling pathways. Ketamine blocks the NMDAR with profound effects on downstream signaling cascades. Unlike monoamine reuptake inhibitor (SSRI) Ketamine improves depressive symptoms within hours and is particularly effective in patients suffering from TRD. Psychomimetic side effects have so far prevented Ketamine's routine use in the clinic as a first-line drug. In order to develop alternative fast-acting drugs with a similar mode of action on the glutamatergic system, but with fewer side effects, a detailed understanding of the molecular events elicited by Ketamine treatment is essential. Alterations affecting the metabolome are a reflection of modified pathway activities in response to drug treatment. In the present study C57BL/6 mice were treated with a single injection of Ketamine with the aim of identifying hippocampal cellular pathway alterations and biomarker candidates. In addition, we have compared affected pathways and biomarkers between Ketamine and SSRI treatment. We applied a targeted polar metabolomics profiling platform and studied the time course of metabolite level changes and their contribution to the antidepressant-like effect of Ketamine. *In silico* pathway analyses revealed profound alterations of several hippocampal pathways. Interestingly, already two hours after a single injection of Ketamine many TCA cycle metabolites were significantly altered and 14 hours after Ketamine treatment most of the glycolytic metabolites were

significantly downregulated. Both these pathways are also affected by chronic the SSRI Paroxetine, albeit 24 days after treatment. This study provides new insights into Ketamine's mode of action and sheds light on pathways that are affected downstream of the NMDAR.

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Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.03/Z36

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Hundred Talent Program of Chinese Academy of Sciences (ZW)

Title: Large-scale network effects of sub-anesthetic ketamine on macaques: New insight into the glutamatergic system for mood disorders

Authors: Q. LV[1], L. YANG[1], Z. WANG[1], G. LI[2], Z. SHEN[1], W. YU[1], Q. JIANG[1], B. HOU[1], J. PU[1], H. HU[1], *Z. WANG[1]
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Abstract: Recently a single sub-anesthetic dose of ketamine has been shown to produce a rapid (within hours) antidepressant response that lasts for 1-2 weeks, which is extremely attractive for novel pharmacological strategy. Although preclinical studies have begun to investigate the various signaling pathways of NMDA receptor blockade, what are the relevant brain circuits that underlie the fast and efficacious action of this agent remains largely unknown. To systematically identify the global network change, we combined the resting-state functional magnetic resonance imaging (fMRI) and graph theoretical analysis to quantitatively examine the topological features of the coherent brain activity. In a randomized, placebo-controlled, crossover design, healthy macaque monkeys (n = 9) received a single-dose administration of saline and ketamine (0.5 mg/kg) respectively, spaced at least 1-month apart. Resting-sate fMRI scans were scheduled ~18 hours after the agent administration and conducted on a Siemens Trio 3T scanner with an enhanced gradient insert AC88. We observed significant difference in global small-world metrics between ketamine and saline groups. Both characteristic path length ($p < 0.001$) and the normalized characteristic path length ($p < 0.01$) of the brain network were increased by ketamine, while the clustering coefficient ($p < 0.01$), local efficiency and global efficiency were

significantly decreased ($p < 0.001$). Nodal strength and network-based connectivity analysis revealed large-scale down-regulation of functional connectivity in the monkey brain after single-dose administration of ketamine, prominently at several key structures in the cortico-striatal-limbic pathways. This finding suggests key targets in the brain reward circuitry that may underlie the rapid, efficacious therapeutic outcome of ketamine.

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Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.04/AA1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: PK profiles of ketamine dosing regimens used in preclinical studies of its anti-depressant-like action

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Abstract: Ketamine is a non-competitive, non-subtype selective *N*-methyl-D-aspartate (NMDA) receptor antagonist. In addition to its well-known use as an anesthetic and analgesic, sub-anesthetic doses of ketamine have also been reported to produce rapid (≤ 2 hrs) and sustained (for days) antidepressant effects in depressed patients. It is also effective in a variety of antidepressant-sensitive behavioral tests and experimental models of depression. In order to better understand the mechanisms through which ketamine's rapid antidepressant effects are mediated, we conducted a pharmacokinetic study in rat using a dose range frequently used in preclinical mechanistic studies. We administered (±) ketamine subcutaneously at 1, 5 and 10 mg/kg as well as intravenously at 1 and 5 mg/kg, sampling plasma at 5 min through 8 hours for subcutaneous administration and 2 min through 8 hours for intravenous administration. Ketamine displayed a shorter half-life and conversion rate to the metabolite, norketamine, in rat compared to published data in human. C_{max} after subcutaneous administration at 1 mg/kg was 189 ng/mL

(0.8 μM), 730 ng/mL (3.1 μM) at 5 mg/kg and 2399 ng/mL (10.1 μM) at 10 mg/kg. C_{max} after intravenous administration was 377 ng/mL (1.6 μM) at 1 mg/kg and 2784 ng/mL (11.7 μM) at 5 mg/kg. Comparing the C_{max} observed in these studies, only the 1 mg/kg, s.c. group was near the estimated C_{max} of ~183 ng/mL (0.8 μM) reported by Zhao et al. (2012) following 40-minute infusion of 0.5 mg/kg ketamine in patients with treatment-resistant bipolar depression. Based on the literature and our own internal observations, 1 mg/kg, (whether s.c. or i.p.) generally is ineffective at producing anti-depressant-like efficacy in preclinical studies; whereas, 2.5 mg/kg or higher (frequently 10 mg/kg) is generally required. From these data, we conclude that at Cmax concentrations achieved in most preclinical studies demonstrating anti-depressant-like efficacy, significant activity at targets besides NMDA receptors, including dopamine D2 receptors, nicotinic receptors and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, may contribute to the observed effect. Based on the available information published on the brain:plasma ratios in rodents and humans (~3-6:1) together with the differences in plasma C_{max}, the dose regimens used in most preclinical studies also likely have activity at additional brain targets besides NMDA receptors compared with the likely brain targets affected in human studies with ketamine.

Disclosures: **W.A. Eckert:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **J.R. Shoblock:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **J.E. McDuffie:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **B.P. Scott:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **M.A. Letavic:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **J. Vegas:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **T. Crowley:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **X. Jiang:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **P. Zannikos:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **J.B. Singh:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C. **G. Chen:** A. Employment/Salary (full or part-time):; Janssen Research & Development, L.L.C..

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.05/AA2

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Acute ketamine treatment induces long lasting behavioral effects in anxio-depressive mice

Authors: *A. M. GARDIER, I. MENDEZ-DAVID, T. PHAM, D. DAVID
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Abstract: Ketamine, a non competitive, glutamatergic NMDA receptor antagonist that binds to the phencyclidine site within this ionotropic Ca2+ channel, displays a rapid antidepressant effect: several double-blind placebo-controlled clinical trials suggested that a single ketamine dose (a 0.5 mg/kg intravenous dose < its anesthetic effect) induced a rapid (within 72 hrs after ketamine) and persistent (for 1 week) antidepressant activity in treatment-resistant depressed patients. This rapid antidepressant response may require an increase in mammalian Target of Rapamycin (mTOR)-dependent expression of BDNF in the prefrontal cortex. Measurements of ketamine effects in animal models of depression (chronic mild stress; chronic corticosterone administration) have been sparse. Most of the studies used animal tests of the antidepressant-like activity (Forced Swim Test, FST for example). Sub-anesthetic ketamine dose (3 mg/kg or below) caused this response in mice. However, some negative results were obtained after acute ketamine injection (0.5 to 12.5 mg/kg) in naïve, non-stressed rodents. Firstly, we performed a full characterization of the putative anxiolytic/antidepressant-like activity of ketamine (10 mg/kg, i.p) in male BALB/cJ mice 24 hours after its administration using the Open Field (OF), Elevated Plus Maze (EPM), Novelty Supressed Feeding (NSF), FST and Splash Test. Male BALB/cJ mice were used for this behavioral study because of their high sensitivity to acute and chronic anxiolytic and antidepressant treatment. Unlike fluoxetine (18 mg/kg, i.p., 24 hours before testing), ketamine induced a trend toward an increase in anxiolytic-like effect in the OF and EPM paradigms. Interestingly, ketamine induced a strong antidepressant-like activity in the NSF and FST. The increase in swimming duration was responsible for this antidepressant-like activity. Changes in neurotransmitters release is currently under investigations using intracerebral *in vivo* microdialysis in mice to depict the mechanism underlying these behavioral effects at 24hr after ketamine injection. Then, we studied the behavioral and neurogenic effects of an acute ketamine dose in a mouse model of an anxiety/depressive-like state induced by chronic CORT administration. Behavioral tests were performed at 7 and 14 days after administration of ketamine (10 mg/kg, i.p.). At 7 days after treatment, but not 14 days, ketamine reduced the immobility duration in the TST and the latency to feed in the NSF paradigm in CORT-treated mice. Overall, our results are in favor of an antidepressant-like response of ketamine lasting for 7 days after its acute administration in anxio-depressive mice.

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Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

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Program#/Poster#: 425.06/AA3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH T32 NS069562-04

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Brain & Behavior Research Foundation

International Mental Health Research Organization

Title: Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses

Authors: *E. S. GIDEONS, E. T. KAVALALI, L. M. MONTEGGIA
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Abstract: Ketamine is a N-methyl-D-aspartate receptor (NMDAR) antagonist that elicits rapid antidepressant responses in patients with treatment-resistant depression. However, ketamine can also produce psychotomimetic effects which limits its utility as an antidepressant raising the question whether the clinically tolerated NMDAR antagonist memantine possesses antidepressant properties. Despite its similar potency to ketamine as an NMDAR antagonist, clinical data suggest that memantine does not exert rapid antidepressant actions for reasons that are poorly understood. In this study we recapitulated the ketamine and memantine clinical findings in mice showing that ketamine but not memantine has antidepressant-like effects in behavioral models. Using electrophysiology, we show that ketamine and memantine effectively block NMDAR-mediated mEPSCs in the absence of Mg^{2+}. However, in physiological levels of extracellular Mg^{2+} we identified key functional differences between ketamine and memantine in their ability to block NMDA receptor function at rest. This differential effect of ketamine and memantine extends to intracellular signaling coupled to NMDAR at rest, in that memantine does not inhibit the phosphorylation of eukaryotic elongation factor-2 nor augment subsequent expression of brain-derived neurotrophic factor, critical determinants of ketamine mediated antidepressant efficacy. These results demonstrate significant differences between the efficacies of ketamine and memantine on NMDA receptor mediated neurotransmission that impacts

downstream intracellular signaling which we hypothesize is the trigger for rapid antidepressant responses. These data provide a novel framework on the necessary functional requirements on NMDA receptor mediated neurotransmission as a critical determinant necessary to elicit rapid antidepressant responses.

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Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

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Program#/Poster#: 425.07/AA4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Research Fund of IRP, NIMH, NIH.

Title: The mood stabilizer lithium ameliorates oxidative stress associated with acute ketamine-induced antidepressant-like effects

Authors: *C.-T. CHIU, L. SCHEUING, H.-M. LIAO, D.-M. CHUANG
NIMH, NIH, Bethesda, MD

Abstract: Numerous clinical and preclinical studies have underscored ketamine's remarkable antidepressant effects. Nevertheless, given the inevitable relapse of depressive symptoms and the potential for abuse, ketamine is not a viable long-term clinical option. The mood stabilizer lithium is a glycogen synthase kinase-3 (GSK-3) inhibitor with strong anti-suicidal properties. Our previous study found that the antidepressant-like effects induced by a single injection of ketamine in a mouse stressed model were potentiated and prolonged by pre- and post-ketamine lithium treatment, respectively. In the present study, we further identified that these behavioral benefits of lithium treatment were associated with GSK-3 inhibition and stimulation of the mammalian target of rapamycin (mTOR)/brain-derived neurotrophic factor (BDNF) signaling pathways in the prefrontal cortex of mouse brain. In addition, we observed that acute injection with ketamine at a dose that produced an antidepressant-like effect (50 mg/kg, i.p.) also markedly increased lipid peroxidation (141.49 ± 7.29%, $P<0.01$), catalase activity (120.64 ± 5.79%, $P<0.01$), and levels of oxidized glutathione (130.90 ± 7.75%, $P<0.01$; compared with stressed control) in the same brain region 20 minutes after injection, indicating elevated oxidative stress. Similar results were also found in the hippocampus and striatum of stressed mice. To investigate whether lithium can protect against the oxidative stress induced by

ketamine, mice were pretreated with 1200 mg/l of lithium chloride in drinking water for three weeks before ketamine challenge. The serum concentration of lithium after three weeks of treatment with this dose was at the lower end (0.483 ± 0.052 mEq/l; n=8) of the therapeutic spectrum for human patients with bipolar disorder. Compared with control stressed mice, lithium pretreatment robustly suppressed ketamine-induced lipid peroxidation (89.35 ± 13.09%, $P<0.01$), catalase activity (85.16 ± 3.40%, $P<0.01$), and levels of oxidized glutathione (103.49 ± 7.73%, $P<0.01$) in the prefrontal cortex of stressed mice. These oxidative metabolism markers induced by ketamine in the hippocampus and striatum were also reduced by lithium pretreatment. Despite the extant evidence suggesting that lithium has antioxidant properties, our study is the first to demonstrate that the oxidative stress induced by ketamine can be completely blocked by lithium pretreatment, underscoring lithium's neuroprotective aspects. Taken together, the antioxidant effects of lithium provide an additional benefit and rationale for its adjunctive use with ketamine in the treatment of depression.

Disclosures: **C. Chiu:** None. **L. Scheuing:** None. **H. Liao:** None. **D. Chuang:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.08/AA5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant R03AA022479

Title: Ketamine prevents alcohol-induced depressive-like behavior in rats

Authors: *O. O. KALEJAIYE, J. FORD, Y. TIZABI
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Abstract: A strong positive association exists between heavy drinking (i.e., alcoholism) and depression, and epidemiological studies suggest that this depression can negatively influence successful cessation of alcohol use. Therefore, there is a fundamental need to develop efficient and fast acting antidepressants that can specifically treat depression associated with alcohol use. Ketamine, a glutamatergic NMDA receptor antagonist has shown promise as quick acting antidepressant. However, its efficacy in alcohol-induced depression is unknown. In this study we sought to provide some answers by subjecting 4 groups of adult male Wistar rats to 4 different treatments. One group was injected with saline as control and all 3 other groups were injected

with alcohol (1g/kg ip) for 7 days to induce depressive-like behavior as assessed by forced swim test (FST) and sucrose preference test (SPT). One group of alcohol-treated rats was injected with 2 mg/kg ketamine daily, half h before alcohol administration and another group of alcohol-treated rats was injected with a single dose of ketamine (2 mg/kg), 16 h after the last alcohol injection. In all cases behavioral tests were carried out approximately 18 h after the last alcohol administration. All animals were tested for general locomotor activity in an automated activity-monitoring cage, for 5 min, followed by FST and SPT. FST, conducted for 5 min, was videotaped and immobility score, reflective of helplessness was determined. Sucrose consumption was performed for 6 h, where the bottles were switched after 3 h to avoid side preference. A decrease in sucrose consumption is reflective of anhedonia (lack of pleasure). Results indicate a "depressogenic" effect of chronic alcohol treatment as evidenced by higher immobility in the FST and a decrease in sucrose consumption. Pretreatment with daily ketamine, or a single injection of ketamine after daily alcohol administration normalized changes induced by alcohol. Locomotor activity was not affected by any treatment. These preliminary results suggest the usefulness of ketamine in alcohol-related depression. Supported by: NIH/NIAAA R03AA022479

Disclosures: O.O. Kalejaiye: None. **J. Ford:** None. **Y. Tizabi:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.09/AA6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Involvement of serotonergic system in the antidepressant-like effects of a metabotropic glutamate 2/3 receptor antagonist and ketamine

Authors: K. FUKUMOTO, M. IIJIMA, *S. CHAKI
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Abstract: Metabotropic glutamate 2/3 (mGlu2/3) receptor antagonists and an N-methyl-D-aspartate (NMDA) receptor antagonist, ketamine have been reported to exert antidepressant-like effects via α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor stimulation and the subsequent stimulation of tropomyosin-related kinase B and the mammalian target of rapamycin signaling pathway, which may lead to an increase in the synthesis of synaptic proteins in animal models. Thus, AMPA receptor stimulation may trigger similar

neuronal mechanisms of antidepressant-like effects of mGlu2/3 receptor antagonists and ketamine. AMPA receptor stimulation has also been shown to mediate an increase in extracellular level of serotonin (5-HT) in the medial prefrontal cortex by an mGlu2/3 receptor antagonist and an NMDA receptor antagonist in rats. However, involvement of serotonergic system in the antidepressant-like effects of mGlu2/3 receptor antagonists and ketamine is not well understood. Therefore, we investigated involvement of serotonergic system in the antidepressant-like effects of an mGlu2/3 receptor antagonist, 2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid (LY341495) and ketamine using animal models of depression such as a forced swimming test (FST) and a novelty-suppressed feeding test (NSFT) of male C57BL/6J mice. The administration of LY341495 or ketamine exerted antidepressant-like effects in the FST and NSFT, which were attenuated by an AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonamide (NBQX). The antidepressant-like effects of LY341495 and ketamine were blocked by pretreatment with a tryptophan hydroxylase inhibitor, para-chlorophenylalanine in the FST and NSFT. Moreover, the effects of LY341495 and ketamine were blocked by a 5-HT1A receptor antagonist, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridynyl) cyclohexane-carboxamide (WAY100635), but not by a 5-HT2A/2C receptor antagonist, ritanserin in the NSFT. These effects of LY341495 and ketamine were mimicked by an AMPA receptor potentiator, 2,3-dihydro-1,4-benzodioxin-7-yl-(1-piperidyl)methanone (CX546) in the NSFT. These results suggest that AMPA receptor-dependent activation of serotonergic system may be involved in the antidepressant-like effects of an mGlu2/3 receptor antagonist and ketamine.

Disclosures: **K. Fukumoto:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd. **M. Iijima:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd. **S. Chaki:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co., Ltd..

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

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Program#/Poster#: 425.10/AA7

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH090067

NIH Grant MH082933

Title: Involvement of the ventral hippocampus in the antidepressant-like effect of ketamine: Behavioral and optogenetic studies

Authors: *F. R. CARRENO, M. DEGUZMAN, A. SHAH, D. J. LODGE, A. FRAZER
Univ. Texas Hlth. Sci. Ctr-San Antonio, San Antonio, TX

Abstract: A single sub-anesthetic dose of ketamine exerts fast-acting and sustained antidepressant effects. However, the mechanisms and brain regions contributing to this effect are still unclear. We examined the role of the ventral hippocampus (vHipp) in the antidepressant response to ketamine as well as the contribution of the neurotrophin receptor TrkB, which is known to be phosphorylated by ketamine administration. Inactivation of the vHipp by lidocaine prevented the sustained (7 days), but not acute (30 min), antidepressant-like effect of ketamine in the forced swim test (FST). Given the role of BDNF in the antidepressant response to ketamine, we examined whether a transient increase in TrkB phosphorylation in the vHipp contributed to the sustained antidepressant response to ketamine. Similar to the effects of intra-vHipp lidocaine, blockade of TrkB receptor phosphorylation (by the tyrosine kinase inhibitor, K252a) in the vHipp at the time of ketamine administration attenuated its antidepressant-like effects measured 1 week following administration. Thus, it appears that ketamine induces plastic changes in vHipp afferents that underlie a sustained antidepressant response. A significant limbic projection of the vHipp is to the mPFC. To elucidate the consequence of augmented vHipp-mPFC activity, we utilized an optogenetic approach. AAV2-hsyn-ChR2-EYFP (or control) was injected into the vHipp and bilateral fiber optic cannulas implanted into the mPFC. ChR2 was activated by laser light and the behavioral effect in the FST recorded. Consistent with our ketamine data, optogenetic stimulation of vHipp-mPFC pathway significantly increased climbing in the FST; however, a decrease in immobility was not observed due to a significant decrease in swimming behavior. To test if the decrease in swimming was due to a feedback pathway from the mPFC to the dorsal raphe nucleus (DRN), we examined optogenetic stimulation following administration of the GABA-A receptor antagonist, bicuculline, into the DRN. Pretreatment with bicuculine abolished the decrease in swimming observed when the mPFC was stimulated and now a significant decrease in immobility was revealed. In conclusion, these data demonstrate a role for vHipp-mPFC plasticity in the antidepressant-like effects of ketamine.

Disclosures: F.R. Carreno: None. **M. DeGuzman:** None. **A. Shah:** None. **D.J. Lodge:** None. **A. Frazer:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.11/AA8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Enantioselective effect of ketamine metabolites on serine racemase expression and function in 1321N1 and PC-12 cells

Authors: N. S. SINGH[1], R. K. PAUL[1], M. KHADEER[1], *L. TOLL[2], I. W. WAINER[1]
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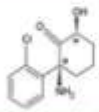
Abstract: (R,S)-Ketamine (Ket) is used in the treatment of bipolar depression (BD) and its clinical effects have been associated with attenuation of NMDA activity and neurogenesis via induction of the mammalian target of rapamycin (mTOR). Ket is rapidly and extensively metabolized into a variety of N-demethylated and hydroxylated compounds. We have recently demonstrated that in the Wistar rat, (R,S)-norketamine and (2S,6S)-hydroxynorketamine stimulated the activating phosphorylation of mTOR and its downstream targets and in PC-12 cells increased the expression of the monomeric form of serine racemase (mSR) via the mTOR pathway while decreasing mSR function as determined by intracellular levels of D-serine (Paul, et al., 2014, Anesthesiology, In press). In this study we have examined the effect of the individual enantiomers of four of the major Ket metabolites on mSR expression and intracellular D-serine levels in PC-12 cells, Table 1. All of the test compounds decreased intracellular D-serine concentrations by ~25% and increased the expression of m-SR. This apparent contradiction will be discussed during the presentation. In all cases the compounds with a 2S-configuration were more active than the corresponding 2R-isomers and the presence of a hydroxyl moiety at carbon 6 in cyclohexanone ring increased potency. These results are consistent with the reported enantiospecific therapeutic differences between (S)-Ket and (R)-Ket and support the hypothesis that ketamine metabolites contribute to the clinical effect of Ket via an indirect mechanism.

Table 1 - The effect of ketamine metabolites on the function and expression of serine racemase (SR) in PC-12 cells where IC_{50} values were determined from the concentration-dependent decrease in intracellular concentrations of D-serine and the arrows represent an increase in the monomeric SR expression relative to untreated control. The relative enantioselectivity factor (α) was determined from the ratio of IC_{50} values derived from the compounds with an R configuration at carbon 2 divided by the IC_{50} values derived from the corresponding compound with an S configuration at carbon 2.

Compound	Isomer	PC-12		Selectivity Factor (α)
		Intracellular D-Ser Concentration – IC_{50}	m-SR expression	
 Nodeketamine	R	224 ± 30 nM	↑	1.94
	S	115 ± 27 nM	↑	
 Dehydroxynorketamine	R	85 ± 30 nM	↑	2.13
	S	40 ± 15 nM	↑	
 Hydroxynorketamine	(2R,6R)	0.65 ± 0.53 nM	↑	4.64
	(2S,6S)	0.14 ± 0.09 nM	↑	
	(2R,6S)	3.76 ± 0.93 nM	↑	1.39
	(2S,6R)	2.69 ± 0.63 nM	↑	

*represents chiral center

Disclosures: **N.S. Singh:** None. **L. Toll:** None. **I.W. Wainer:** None. **R.K. Paul:** None. **M. Khadeer:** None.

Poster

425. Mood Disorders: Ketamine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 425.12/AA9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Fondation de Prefargier

Title: Determining the involvement of lactate in mediating antidepressant responses

Authors: ***M. ELSAYED**[1,2], J.-M. PETIT[1,2], V. ELIGERT[1,2], P. J. MAGISTRETTI[1,2,3]
[1]UNIL, Lausanne, Switzerland; [2]Brain Mind Inst., EPFL, Lausanne, Switzerland; [3]BESE division, KAUST, Thuwal, Saudi Arabia

Abstract: Studies from our laboratory have shown that intracortical injection of Lactate, but not other metabolic products, stimulates the expression of plasticity related-genes in mice.

Furthermore, we have also shown that antidepressants can upregulate the expression of growth factors and promote Lactate release from astrocytes *in vitro* (Allaman et al, 2011). Given that patients with depression show decreases in astrocyte densities and in neuroplasticity, we ask whether Lactate, a glial metabolic product is involved in mediating antidepressant responses. Initial experiments were carried out to determine if antidepressants increase brain lactate levels *in vivo*. We used specific lactate biosensors implanted in freely-moving adult mice. With this technique, we determined the influence of different classes of antidepressants on brain lactate levels. Our preliminary findings indicate that unlike conventional antidepressants whose effects were examined after a chronic injection, the rapid acting antidepressant ketamine resulted in an increase in extracellular lactate levels within hours of injection. Moreover, this effect is specific to ketamine's action within the brain, as there was no influence on lactate levels in the blood. Future experiments would need to characterize the involvement of Lactate in ketamine mediated antidepressant effects. Furthermore, given that these biosensors are limited to recordings of changes in concentration following manipulation, additional related assays such as microdialysis or magnetic resonance spectroscopy would be needed to be carried out to confirm the specificity of the effects of the different classes of antidepressants on brain lactate levels.

Disclosures: M. Elsayed: None. **J. Petit:** None. **V. Eligert:** None. **P.J. Magistretti:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.01/AA10

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: SSRI induced endogenous BDNF expression in Dentate Gyrus precisely regulates adult neurogenesis and anti-depressant effect

Authors: *Z. MA[1], L. F. PARADA[2]
[1]Developmental Biol., UT Southwestern Med. Ctr., Dallas, TX; [2]Developmental Biol., UT Southwestern Med. Ctr. at Dallas, Dallas, TX

Abstract: Hippocampal neurogenesis has been shown to play an important role in the behavioral repsonse to anti-depressant drugs (ADs) such as selective serotonin reuptake inhibitors (SSRIs). Despite rapid neurochemical action, SSRIs usually take several weeks to exert their clinical efficacy, suggesting a potential time lapse between the activation of neurotrophic factors and their actions on hippocampal neurogeneis. Our previous studies have demonstrated that chronic

SSRI treatment specifically causes trkB activation leading to enhanced progenitor cell proliferation and neurogenesis in Dentate Gyrus (DG). Specific ablation of TrkB in these progenitors blocks proliferation, neurogenesis and behavioral response to SSRIs. However, it is still not clear that how different cell types in DG respond to SSRI treatment and increase adult neurogenesis through BDNF transcriptional activation. Here, by using in-situ hybridization and BDNFlox-lacZ mice, in which lacZ gene is controlled by endogenous BDNF promoters, we found both granule cells and neural stem/progenitor cells increase BDNF expression with the exposure to chronic SSRI treatment, and induced BDNF proteins are mainly derived from differentiated DG granule neurons. Although the specific ablation of trkB in neural progenitor cells by Nestin-creER^{t2} can totally abolish the behavioral response to anti-depressive treatment, mice lacking BDNF in the same cell types initially respond normally to chronic ADs one week after the last dose, and then display the behavioral insensitivity in depression- and anxiety-like paradigms after two weeks. In contrast, mice with BDNF specifically knocking-out in DG neurons dramatically decrease BDNF level in DG and do not show any anti-depressant response to ADs. Moreover, it seems that the status of adult neurogenesis in DG is well correlated with the achievement of behavioral response to ADs in these BDNF/trkB conditional knock-out lines. More detailed analyses of neurogenesis are still going-on. Thus, our current data suggests that endogenous BDNF is an important mediator for hippocampal neurogenic niche to precisely regulate chronic anti-depressant response in mice.

Disclosures: Z. Ma: None. **L.F. Parada:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.02/AA11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Exploring antidepressant-induced affective blunting in rats: Assessment of chronic fluoxetine administration on operant responding

Authors: *K. MATAZEL[1], R. J. RICE[1], A. L. PEHRSON[2], A. J. PRUS[1]
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Abstract: The most frequently prescribed pharmacological agents used for treating depression are those that prevent the reuptake of serotonin. In addition to the commonly known adverse effects of these compounds (e.g., sexual dysfunction, insomnia, serotonin syndrome), patients

chronically treated with serotonin reuptake inhibitors may develop a flattened affective state (sometimes referred to as “affective blunting”) characterized by a lack of facial emotional expression, little intonation or vocal variation, and decreased communicative gesturing that differ from depressive symptomology. The purpose of this study was to determine if antidepressant drugs may elicit an affective blunting state in rats by assessing the effects of repeated administration of the selective serotonin reuptake inhibitor fluoxetine on lever pressing for positive reinforcement. After response rates stabilized, a food deprivation curve was conducted over a 24 hour period before and after 21 days of fluoxetine administration (16 g/L in home cage water bottles). Fluoxetine administration did not produce a change in response rates compared to baseline. Further studies are needed to evaluate different types of positive reinforcers (e.g., sucrose pellets) and to assess avoidance of aversive stimuli (e.g., bright light) after chronic fluoxetine administration, in order to determine if affective blunting can be modeled in rats.

Disclosures: **K. Matazel:** None. **R.J. Rice:** None. **A.L. Pehrson:** None. **A.J. Prus:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

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Program#/Poster#: 426.03/AA12

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: IMI grant n° 115008 - NEWMEDS

Title: Serotonin transport occupancy by S-citalopram and R/S-citalopram in the nonhuman primate brain: A [11C]MADAM PET study

Authors: *S. J. FINNEMA[1], C. HALLDIN[1], B. BANG-ANDERSEN[2], C. BUNDGAARD[2], L. FARDE[1]
[1]Dept. of Clin. Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Neurosci. Drug Discovery Denmark, H. Lundbeck A/S, Valby, Denmark

Abstract: *Rationale* Positron emission tomography (PET) can be used to assess changes in endogenous neurotransmitter concentrations in the living brain. Very recently, some novel serotonin (5-HT) receptor radioligands have been reported to be sensitive to drug-induced changes in 5-HT concentrations. High doses of selective 5-HT reuptake inhibitors (SSRIs) have been shown to decrease cortical radioligand binding in the monkey brain, consistent with increased 5-HT concentrations (Milak et al, 2011; Ridler et al, 2011; Nord et al, 2013). However,

in two recent studies on healthy subjects, a single, lower and clinical relevant SSRI dose increased cortical radioligand binding suggesting a potential decrease in 5-HT concentration in projection areas when initiating SSRI-treatment (Selvaraj et al, 2012; Nord et al, 2013). *Objectives* The cross-species differential SSRI effect may be explained by SSRI-induced 5-HT transporter (SERT) occupancy in the monkey brain being higher than clinical relevant. We here determined SERT occupancy after single doses of *S*-citalopram or *R*/*S*-citalopram in the monkey brain. *Methods* Ten PET measurements with [^{11}C]MADAM were performed in two female cynomolgus monkeys prior and post *S*-citalopram or *R*/*S*-citalopram administration. Relationships between dose, plasma concentration and SERT occupancy were interpreted by one-site saturation analysis. The binding affinity was expressed as the dose (ID_{50}) or plasma concentration (K_i) at which 50% SERT occupancy was achieved. *Results* SERT occupancy increased in a dose- and plasma concentration-dependent manner. The estimated ID_{50} and K_i values were 0.038 mg/kg (95%CI = 0.013-0.062) and 18 nmol/L (95%CI = 6.0-30) for *S*-citalopram and 0.046 mg/kg (95%CI = 0.013-0.078) and 7.9 nmol/L (95%CI = 2.2-14) for *R*/*S*-citalopram, respectively. *Conclusions* The obtained K_i values are comparable to values reported for human subjects (Lundberg et al, 2007). It can be concluded that the SSRI doses in previously reported 5-HT release studies in monkeys almost saturated SERT (Milak et al, 2011; Ridler et al, 2011; Nord et al, 2013). The PET-measured cross-species differential effect of SSRI on cortical 5-HT concentration is thus likely related to the SSRI dose, and appears consistent with known SSRI dose-effects on regional differences in microdialysis studies (Invernizzi et al, 1992). Future monkey studies using SSRI doses inducing clinically relevant SERT occupancy are called for to provide further understanding of SSRIs delayed onset of therapeutic effect.

Disclosures: **S.J. Finnema:** None. **C. Halldin:** None. **B. Bang-Andersen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **C. Bundgaard:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **L. Farde:** A. Employment/Salary (full or part-time):; AstraZeneca Pharmaceuticals.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.04/AA13

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Tricyclic antidepressant amitriptyline acts on astrocytes leading to the increase in the FGF2 expression: A role of early growth response 1 signaling

Authors: N. KAJITANI[1,2], *K. HISAOKA[2], M. OKADA-TSUCHIOKA[1], M. HOSOI[1], C. SHIBASAKI[1,3], N. MORIOKA[2], Y. NAKATA[2], M. TAKEBAYASHI[1,3]
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Abstract: [Background] Recently, fibroblast growth factor 2 (FGF2) has been proposed to play an important role in the therapeutic action of antidepressants. We have previously reported that a tricyclic antidepressant amitriptyline increases the FGF2 production and its release in rat primary cultured astrocytes, which is a primary source of FGF2 in the brain. However, the intracellular signaling of the amitriptyline-induced FGF2 production in astrocytes remains unclear. In this study, we focused on early growth response 1 (EGR1), which is one of the transcription factor known to regulate the FGF2 gene expression, and we examined whether the EGR1 is involved in the amitriptyline-induced FGF2 expression in astrocytes. [Method] We prepared astrocytes from the cortex of 1-day-old neonatal Wistar rat. We measured the expression levels of EGR1 and FGF2 mRNA using real time PCR and protein levels using immunoblotting. [Results] Treatment with amitriptyline significantly increased the EGR1 production in rat primary cultured astrocytes. We used the siRNA for EGR1 to examine the role of EGR1 in the amitriptyline-induced FGF2 expression in astrocytes. Down-regulation of EGR1 using siRNA blocked the amitriptyline-induced FGF2 mRNA expression. MEK1/2 inhibitor PD98059 significantly inhibited both EGR1 and FGF2 mRNAs induced by amitriptyline. Actually, amitriptyline increased the phosphorylation of ERK1/2 in astrocytes, suggesting that amitriptyline activates the ERK/EGR1 signaling cascade, resulting in FGF2 production. Furthermore, we attempted to identify the mechanism through which amitriptyline activates ERK/EGR1/FGF2 signaling cascade in astrocytes. Epidermal growth factor receptor (EGFR) inhibitor AG1478 and FGF receptor (FGFR) inhibitor SU5402, but not platelet-derived growth factor inhibitor AG1296 inhibited the amitriptyline-induced intracellular signaling cascade. In addition, heparin and matrix metalloproteinase (MMP) inhibitors GM6001 and prinomastat inhibited the effects of amitriptyline. [Conclusion] These results suggest that the amitriptyline-induced FGF2 expression is involved in the ERK-dependent EGR1 production. EGFR and/or FGFR mediate this intracellular signaling. Amitriptyline-induced EGFR and FGFR signaling might occur, at least in part, by the MMP-dependent shedding of heparin-sensitive EGFR and/or FGFR ligands, thus resulting in FGF2 induction in astrocytes. These findings indicate a novel mechanism of action of amitriptyline through astrocytes and further suggest that targeting this mechanism could lead to the development of a new class of antidepressants.

Disclosures: **N. Kajitani:** None. **K. Hisaoka:** None. **N. Morioka:** None. **M. Hosoi:** None. **Y. Nakata:** None. **M. Okada-Tsuchioka:** None. **C. Shibasaki:** None. **M. Takebayashi:** None.

Poster

426. Mood Disorders: SSRIs

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: CREST, JST

Title: Transcriptomic evidence for immaturity in the frontal cortex of mice treated with antidepressants

Authors: *K. OHIRA, H. HAGIHARA, R. TAKEUCHI, T. MIYAKAWA
Div. Systems Med. Sci, ICMS, Fujita Hlth. Univ., Toyoake, Japan

Abstract: The selective serotonin reuptake inhibitor, fluoxetine (FLX), is widely used to treat depression and anxiety disorders. Previous studies have demonstrated that FLX treatment can reverse the established neuronal maturation of granule cells in the hippocampal dentate gyrus, and of inhibitory interneurons in the basolateral amygdala. FLX can also increase hippocampal adult neurogenesis, which is the most widely reported effect. Recently, using immunohistological methods, we found that chronic FLX treatment decreased the levels of parvalbumin, which could be used as a maturation marker of fast-spiking interneurons, and perineuronal nets (PNNs), which are formed mainly around mature fast-spiking interneurons, in the medial frontal cortex (mFC) of adult mice, suggesting that FLX induces pseudo-immature status of these types of neurons. In this study, to further characterize the effect of FLX on mFC, we compared genome-wide gene expressions of FLX-treated mFC with those of the corresponding region of normally developing brains in mice. We found that the mFC in adult mice treated with FLX resembled juvenile mFC in terms of genome-wide expression profile. The common gene alterations between FLX-treated mFC of adult mice and mFC of normally developing mice might be partly accounted for by maturational abnormalities in fast-spiking interneurons, astrocytes, and oligodendrocytes, although the contributions of other cell types to the transcriptomic immaturity of the mFC treated with FLX cannot be excluded. In addition, gene expression patterns in the FLX-treated mFC of adult mice were similar to those in the developing mFC of humans. Our results suggest that FLX treatment induces pseudo-immaturity of the mFC of adult mice with respect to gene expression patterns.

Disclosures: **K. Ohira:** None. **H. Hagihara:** None. **R. Takeuchi:** None. **T. Miyakawa:** None.

Poster

426. Mood Disorders: SSRIs

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH/FIC R21MH095656 to MAG

Title: Cognitive assessments prospectively differentiate future responders and non-responders to selective serotonin reuptake inhibitor treatment among medication-naïve patients with major depressive disorder

Authors: *M. M. HERZALLAH[1,2], M. B. TAHA[2], J. Y. NATSHEH[2,3], M. A. SEHWAIL[2], M. A. GLUCK[3]
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Abstract: Short cognitive assessments of learning from positive feedback have the potential to prospectively differentiate future SSRI responders from non-responders in patients with Major Depressive Disorder (MDD). We evaluated a group of medication-naïve patients with MDD before and after 4-6 weeks of treatment with SSRI, as well as a matched group of controls tested across the same time interval. All subjects were administered a probabilistic category-learning task that allowed for the dissociation of learning from positive versus negative feedback. At the time of retesting, 65% of our sample responded to SSRI treatment, while 35% were non-responders. We compared the cognitive profiles of these two subpopulations at both baseline and retest against that of healthy subjects. While SSRI non-responders show balanced learning between positive and negative feedback at both baseline and again at retesting following SSRI administration, the SSRI-responders in their medication-naïve state show a strong bias for negative feedback, which was later altered by SSRI therapy to become more balanced during the subsequent post-treatment retesting. The findings in SSRI-responders replicate our earlier results (Herzallah et al., 2013a) using a within-subject design. These cognitive differences between responders and non-responders assessed prospectively prior to any SSRI treatment have the potential to inform the development and validation of a pre-treatment cognitive assessment to predict future clinical response to SSRIs. No such assessment currently exists and, if successfully developed and validated, would have the the potential to significantly impact clinical treatment as well as inform the search for new antidepressant medications that would provide symptomatic relief to those who do not benefit from SSRIs. The ability to differentiate, a priori, the SSRI-

responders from the non-responders prior to initiating antidepressant treatment, would also shed much-needed light on the complex and poorly understood underlying heterogeneity of MDD.

Disclosures: **M.M. Herzallah:** None. **M.B. Taha:** None. **J.Y. Natsheh:** None. **M.A. Sehwail:** None. **M.A. Gluck:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.07/AA16

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Selective serotonin reuptake inhibitor treatment response: Identification of affected molecular pathways in responder and non-responder mouse model

Authors: D. PARK, C. DOURNES, *O. F. ALMEIDA, M. B. MÜLLER-SITZ, C. W. TURCK
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Abstract: Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly used antidepressants for the treatment of psychiatric diseases including Major Depression Disorder (MDD) and various types of anxiety-related disorders. However, current SSRIs have a delayed onset of therapeutic efficacy of several weeks. In addition, a substantial number of patients do not show any improvement during or after antidepressant treatment. To analyze antidepressant treatment resistance, we used the forced swim test (FST) which differentiates responder and non-responder mice. Following one month of chronic Paroxetine treatment DBA mice were categorized into a Paroxetine-treated Long-time Floating (PLF) group, Paroxetine-treated Intermediate Floating (PIF) group, and a Paroxetine-treated Short-time Floating (PSF) group. To identify proteome signatures in PLF and PSF mice, we performed quantitative mass spectrometry of hippocampal membrane- and cytosol-fractions. Metabolite level differences were also investigated in the hippocampus and plasma of PLF and PSF mice. Integrated proteomic and metabolomic analyses revealed that pathways including energy metabolism, neurotransmitter degradation, and glutamatergic signaling are differentially affected in response to chronic Paroxetine treatment in PLF and PSF mice. Validation by Western blot analyses revealed that expression of N-Methyl-D-aspartate (NMDA) receptors 1 (NR1), NR2A and NR2B, post synaptic density-95 (PSD-95) and neuronal nitric oxide synthase (nNOS) is markedly lower in PSF compared to PLF mouse hippocampi and correlates with FST floating time. These data point towards a role of glutamatergic neurotransmission in SSRI treatment response.

Disclosures: **D. Park:** None. **C. Dournes:** None. **O.F. Almeida:** None. **M.B. Müller-Sitz:** None. **C.W. Turck:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.08/AA17

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Chronic fluoxetine induces the enlargement of perforant path synapse in the dentate gyrus: 3D ultrastructural analyses using FIB/SEM

Authors: ***Y. KITAHARA**[1], K. OHTA[2], T. SHUTO[1], M. KUROIWA[1], N. SOTOGAKU[1], H. HASUO[3], A. TOGO[4], K.-I. NAKAMURA[2], A. NISHI[1]
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Abstract: Chronic fluoxetine increases the excitability of mature granule cells (GCs) in the dentate gyrus (DG) as well as the stimulation of neurogenesis. Perforant path axons (boutons) from the entorhinal cortex to DG form synapses on dendritic spines of GCs, known as perforant path synapse. Electrophysiological analysis using voltage sensitive dye detected the enhanced excitatory neurotransmission of perforant path synapse in the middle molecular layer (MML) of DG after chronic fluoxetine treatment. In this study, we investigated the effect of chronic fluoxetine (15 mg/kg/day, 14 days) on the morphology of perforant path synapse in the MML by focused ion beam (FIB)/scanning electron microscopy (SEM). 3D ultrastructural analyses of dendritic spine revealed the appearance of the extremely large-sized spine after chronic fluoxetine, which was confirmed with quantification of spine volume. The large-sized spine had a postsynaptic density with large volume. However, chronic fluoxetine did not affect spine density or volume distribution of the regular-sized spine. We next analyzed characteristics of perforant path bouton. The presynaptic bouton connected to the large-sized spine was large in volume and contained mitochondria and synaptic vesicles with large volume. In addition, the presynaptic bouton with large volume had a connection with multiple spines, and a tight correlation between volume of each presynaptic bouton and sum of spine volumes connected to

the bouton was found. These results demonstrate that chronic fluoxetine treatment affects structures of both the perforant path axon and the dendritic spine of GCs and induces the enlargement of perforant path synapse, which may be involved in the increased glutamatergic neurotransmission of perforant path synapse.

Disclosures: **Y. Kitahara:** None. **K. Ohta:** None. **T. Shuto:** None. **M. Kuroiwa:** None. **N. Sotogaku:** None. **H. Hasuo:** None. **A. Togo:** None. **K. Nakamura:** None. **A. Nishi:** None.

Poster

426. Mood Disorders: SSRIs

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Program#/Poster#: 426.09/AA18

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: VA Merit 1I01CX000501

Title: Diving while taking an antidepressant: A case report of a diver with depression

Authors: D. A. DANCZYK[1], *J. V. PARDO[1], D. F. COLVARD[2]
[1]VAMC, Minneapolis, MN; [2]DivePsych, Raleigh, NC

Abstract: INTRODUCTION / BACKGROUND Very little research has been conducted on antidepressant use in the diver population. The prevalence of divers with depression requiring medications is estimated at 4.8%. The purpose of this article is to assist clinicians in the treatment of Major Depressive Disorder (MDD) for those with occupational or recreational diving habits. MATERIALS / METHODS This is a case presentation of a 37 year old male veteran with MDD, recurrent, who deep-sea dives as a hobby, and required cross-titration from mirtazapine to escitalopram due to weight gain. Data gathered from patient interview as well as treatment record. RESULTS Anecdotal evidence indicates diving is in itself a therapeutic treatment for mild depression. The patient in this case successfully cross-titrated to an SSRI, and did not have side effects while diving. SUMMARY / CONCLUSIONS Being treated with an antidepressant is no longer a strict contraindication to diving. No controlled studies have been conducted of divers at various pressure depths while on psychotropic medication. Despite this, a framework for determining fitness to dive can be used by the clinician. This includes close assessment of side effects before diving in determining the safety of a medication on a case-by-case basis.

Disclosures: **D.A. Danczyk:** None. **J.V. Pardo:** None. **D.F. Colvard:** F. Consulting Fees (e.g., advisory boards); Wake Research Associates, Raleigh NC; I have been a psychiatric consultant in clinical drug trials involving antidepressant medications.. Other; I host the website www.DivePsych.com which provides evidence-based information on stress, anxiety and panic for scuba divers and speak and write on psychiatric aspects of recreational and commercial s, scuba diving internationally. Occassionally, i am compensated for the articles and presentations..

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.10/AA19

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: MH064065

MH070890

HD053000

MH083045

Foundation of Hope for the Research and Treatment of Mental Illness

Title: Neurodevelopment of infants with prenatal SSRI exposure

Authors: ***S. C. JHA**[1], S. MELTZER-BRODY[2], S. WOOLSON[2,3], R. M. HAMER[2,3], M. AHN[3], H. ZHU[3], R. J. STEINER[2], M. STYNER[2,4], J. H. GILMORE[1,2], R. C. KNICKMEYER[1,2]
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Abstract: Selective serotonin reuptake inhibitors (SSRIs) are the most common form of psychotherapy among pregnant women with mood disorders. Serotonin plays a key role in neuronal proliferation, migration, and synaptogenesis, raising concerns about possible effects on the fetal brain. Research on prenatal SSRI exposure and human neurodevelopment has yielded conflicting results and no study has examined this issue using neuroimaging. The aim of this retrospective cohort study was to determine whether brain volumes and white matter microstructure differed in neonates exposed to SSRIs during pregnancy. 27 SSRI-exposed neonates of women with mood disorders were matched to 54 unexposed neonates with no history

of maternal mood disorder. High-resolution structural magnetic resonance imaging (sMRI) and diffusion weighted imaging (DWI) scans were acquired with a 3T Siemens Allegra scanner. An automatic, atlas-moderated expectation maximization segmentation tool was used to classify global tissue volumes. Regional differences in gray matter volumes were examined using deformation morphometry. Quantitative tractography was performed using the UNC-Utah NA-MIC DTI framework adapted for our neonatal sample. No significant differences were found in global tissue volumes. SSRI-exposed neonates exhibited a cluster of decreased gray matter in the thalamus ($p = 0.0088$) compared to unexposed neonates. DTI analysis revealed significantly altered diffusion along major white matter tracts. Results suggest SSRI exposure has minimal effects on tissue volumes. In contrast, SSRI exposure may impact white matter microstructure. Additional research is needed to clarify whether SSRIs directly alter white matter development or whether this relationship is mediated by severity of depressive symptoms during pregnancy.

Disclosures: **S.C. Jha:** None. **S. Meltzer-Brody:** 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.; Astra Zeneca. **S. Woolson:** Other; CeNeRx, Janssen Pharmaceuticals. **R.M. Hamer:** F. Consulting Fees (e.g., advisory boards); Abbott, Allergan, Cenerx, Columbia University, Endo, Lilly, Novartis, Pfizer, Roche, Wyeth. Other; Lundbeck, Sun, Caraco, Forest, Teva, Barr, Mylan, Eurand, Cephalon, Anesta, Marial. **M. Ahn:** None. **H. Zhu:** None. **R.J. Steiner:** None. **M. Styner:** None. **J.H. Gilmore:** None. **R.C. Knickmeyer:** 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.; Pfizer.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.11/AA20

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: F31 MH 101984 (CRT)

RO1ES019583 (AMA)

Title: Fluoxetine treatment ameliorates adult-onset depression induced by perinatal arsenic exposure via a neurogenic mechanism

Authors: *C. R. TYLER, B. SOLOMON, A. M. ALLAN
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Abstract: The role of environmental factors in the etiology of major depressive disorder (MDD) is widely acknowledged, but the molecular mechanisms and responses to antidepressants are relatively unknown. Epidemiological studies have shown that arsenic exposure increases the risk for developing psychiatric disorders, including MDD. In this study, we used a 50 parts-per-billion (ppb) perinatal arsenic exposure paradigm, which produces deficits in adult hippocampal neurogenesis (AHN) at postnatal day (PD) 70, to assess the potentially beneficial effects of chronic fluoxetine (50 or 100 mg/L) treatment on environmentally induced depression. Arsenic exposure generated depressive-like symptoms in a mild learned helplessness (LH) task in the forced swim task after 10 minutes of exposure to 2,4,5-trimethylthiazoline (TMT, as a predator odor). Five weeks of fluoxetine treatment prevented depressive-like behaviors in both the LH and FST in arsenic-exposed animals at PD70. The stress response, measured via corticosterone (CORT) levels at baseline and after TMT exposure, was blunted in arsenic-exposed animals; chronic fluoxetine treatment attenuated this effect. Morphologically, fluoxetine reversed arsenic-induced deficits in the number of differentiated and surviving neural progenitor cells (NPCs). Enhanced neurogenesis after fluoxetine treatment is likely mediated via increased brain-derived neurotrophic factor (BDNF), c-AMP response element binding protein (CREB), the glucocorticoid receptor (GR), or histone deacetylase 2 (HDAC2) in dentate gyrus tissue from arsenic-exposed males. This study demonstrates that damage induced by perinatal arsenic exposure is reversible with chronic fluoxetine treatment resulting in restored resiliency to depression via a neurogenic mechanism.

Disclosures: C.R. Tyler: None. **B. Solomon:** None. **A.M. Allan:** None.

Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.12/AA21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Association between the COMT Val158Met polymorphism and the clinical response to SSRI or SSRI with antipsychotics in obsessive-compulsive disorder

Authors: *H. UMEHARA[1], S. NUMATA[1], A. TAJIMA[2], M. KINOSHITA[1], S. NAKAAKI[3], I. IMOTO[2], S. SUMITANI[1], T. OHMORI[1]

[1]Dept. of Psychiatry, [2]Dept. of Human Genet., The Univ. of Tokushima Grad. Sch., Toskushima, Japan; [3]Lab. of Aging, Behavior and Cognition, Dept. of Neuropsychiatry, Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Object: Accumulating evidence indicates that abnormalities in serotonergic and dopaminergic systems are involved in the pathophysiology and/or treatment of obsessive-compulsive disorder (OCD). Catechol-O-methyltransferase (COMT) is an enzyme that participates in the metabolic inactivation of dopamine and norepinephrine, and the Met allele of the COMT Val158Met polymorphism (rs4680) is associated with lower enzymatic activity. The purpose of the present study was to investigate whether this functional variant of the COMT gene is associated with OCD and the clinical responses of OCD. Methods: We performed a case-control association study between rs4680 and OCD (n=1,115), and conducted a meta-analysis of genetic association studies (n=3,659). Ninety-one patients with OCD participated in our pharmacogenetic study. The patients were divided into three groups according to pharmacological response, as evaluated by the Yale Brown Obsessive-Compulsive Scale (Y-BOCS): group A, responders to a selective serotonin reuptake inhibitor (SSRI) (n=53); group B, responders to a SSRI with an atypical antipsychotic (n=22); and group C, non-responders to a SSRI with an atypical antipsychotic (n=16). Patients who showed a >35% decrease on the Y-BOCS after treatments were considered responders. The risks of OCD and pharmacological response were tested for their association with rs4680 genotypes by logistic regression, assuming an additive contribution of the Met allele. Results: Although we found no significant association between the Met allele and OCD risk within the Tokushima sample set (per-allele OR, 1.15; 95% CI, 0.90-1.48; P = 0.27), the meta-analysis of the genetic association studies, including our sample set, demonstrated a significant association between the Met allele and OCD (per-allele OR, 1.16; 95% CI, 1.05-1.48; P = 0.0054). On the other hand, no significant association between the Met allele and SSRI response (group A vs. group B plus C) was observed (per-allele OR, 1.40; 95% CI, 0.76-2.58; P = 0.28). Moreover, no significant association between the Met allele and the response to a SSRI with antipsychotics (group B vs. group C) was observed (per-allele OR, 0.37; 95% CI, 0.18-1.06; P = 0.06). Conclusion: Our results suggest that the COMT Val158Met polymorphism could be a risk factor for developing OCD. However, this polymorphism may not be associated with the therapeutic responses to a SSRI or to a SSRI with an atypical antipsychotic in OCD.

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Poster

426. Mood Disorders: SSRIs

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 426.13/AA22

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Whitehall Foundation

Title: Region-specific induction of FosB isoforms in mouse brain after stress or chronic fluoxetine exposure

Authors: M. A. THIBAULT[1], A. L. EAGLE[1], S. KASKA[1], E. J. NESTLER[2], M. S. MAZEI-ROBISON[1], V. VIALOU[3], *A. ROBISON[1]
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Abstract: An estimated 1 in 10 U.S. adults report depression, but current pharmacological therapies are effective in only about 50% of patients. In order to uncover novel gene targets that may allow for therapeutic treatment of currently refractive individuals, we examine the gene targets of transcriptional changes that occur with chronic exposure to antidepressants, including the selective serotonin reuptake inhibitor fluoxetine. We have previously shown that induction of the transcription factor ΔFosB in the nucleus accumbens (NAc) of mice promotes resilience to the social defeat model of depression and is required for fluoxetine-mediated reversal of the social defeat phenotype (Vialou et al., 2010). Here, we show by immunohistochemistry that FosB isoforms are induced in more than 25 different brain regions by chronic fluoxetine, including many regions classically associated with depression and mood, i.e. hippocampus (HPC) and prefrontal cortex (PFC). Further, we demonstrate by Western blot that FosB isoforms are differentially expressed by brain region, both in the basal state and after fluoxetine-mediated induction. While HPC and PFC have low basal levels of FosB isoforms and show high levels of ΔFosB after fluoxetine, NAc has a higher basal expression of FosB, ΔFosB, and Δ2ΔFosB, and shows a proportionally greater induction of full-length FosB after fluoxetine. We also observe differential isoform expression in these brain regions after chronic social defeat stress, indicating that FosB isoform expression patterns may underlie susceptibility and resilience to stress. Because each FosB isoform has different gene targets that may very further by brain region, we hypothesize that determining the brain-region-specific gene expression changes mediated by FosB isoforms may uncover novel targets for therapeutic intervention in mood disorders that could improve treatment of currently refractive individuals. In line with this hypothesis, we are currently conducting experiments to determine whether ΔFosB expression in hippocampus controls mood or mediates the antidepressant effects of fluoxetine and are exploring ΔFosB gene targets in this and other brain regions.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.01/AA23

Topic: F.01. Human Cognition and Behavior

Support: Ralph Wilson Family Foundation Team Physician Fund

Title: Cognitive aging in retired professional athletes

Authors: *A. HINDS, J. J. LEDDY, J. BAKER, T. SHARMA, B. S. WILLER
Univ. at Buffalo, Buffalo, NY

Abstract: Recent reports of early onset dementia, cognitive impairment, and violent behavior in individuals with a history of concussive injury has raised concern over the long-term risks for athletes participating in contact sport. Understanding the risk factors and symptom progression that may ultimately lead to a diagnosis of chronic traumatic encephalopathy (CTE) in former professional athletes is essential for early intervention and treatment of affected individuals (Antonius et al., 2014). Previous literature suggests that difficulty with executive function is a problem in athletes at risk for developing CTE (Stern et al., 2011). To evaluate risk for CTE, we compared cognitive performance in 18 retired professional athletes (aged 40-72) that had played a contact sport, with age-matched controls from non-contact sports. Contact athletes included professional hockey and football players who had played in the National Football league and National Hockey league. All participants completed a battery of neuropsychological tests that assess a range of cognitive functions, including memory, attention, language, information processing speed, and executive function. We also assessed self-report of executive functioning (the BRIEF questionnaire), lifestyle factors, mood, and behavioral characteristics such as impulsivity and risk-taking. Although we did not find any specific pattern of cognitive deficits amongst contact sport athletes, more than two-thirds of these individuals self-reported executive dysfunction on the BRIEF, scoring in the abnormal range on indices of behavioral regulation and metacognition (which includes working memory, task monitoring, planning and organizing). Interestingly, self-report of these deficits was not consistent with actual cognitive difficulty, as measured by performance on several tests requiring executive functioning (including the Wisconsin Card Sorting test). These results suggest that, while contact athletes may be at risk for long-term executive function difficulties, we do not find substantial evidence of a direct causal link between sport history and executive dysfunction.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.02/AA24

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Braveheart Foundation

Title: Functional differences in the brain following trauma: A quantitative meta-analysis of fMRI studies in post-traumatic stress disorder

Authors: ***E. A. STARK**[1], C. E. PARSONS[2], A. STEIN[2], H. MCMANNERS[2], A. EHLERS[3], M. L. KRINGELBACH[2]
[1]Univ. of Oxford, OXFORD, United Kingdom; [3]Exptl. Psychology, [2]Univ. of Oxford, Oxford, United Kingdom

Abstract: Neurobiological models of post-traumatic stress disorder (PTSD) propose that dysregulation of limbic-prefrontal activity underlies its aetiology. Much of the empirical data for these theories is derived from neuroimaging studies where the brain activity of adults with PTSD is compared to that of adults without PTSD. However, the typical comparison groups in functional magnetic resonance imaging (fMRI) studies are mixed: some trauma-exposed and some unexposed. We therefore performed a systematic review and voxel-based meta-analysis of task-based fMRI studies of PTSD, to explore differences in functional activity between adults with PTSD and either trauma-exposed or unexposed control participants. We aimed to disentangle the effects of trauma exposure on the brain from effects of trauma exposure leading to PTSD We searched the databases: Web of Science, PsycINFO, and Scopus for studies published between 1st January 2000 and 31st December 2013. Fifty studies were included in the final review. The analysis was threefold. First, we used activation likelihood estimation analysis to explore significant differences in regional brain activity between adults with PTSD and adults without PTSD. Second, we separated studies by the nature of the comparison group: whether they had been exposed to trauma or not. Third, we explored functional differences in the PTSD brain in response to stimuli unrelated to trauma, in comparison to that of both trauma-exposed and trauma-naïve controls. Our main results suggest that the experience of trauma, whether or not it leads to PTSD, may have an important, long-lasting effect upon functional brain activity.

Trauma-exposed individuals who do not develop PTSD do still appear to display functional differences in brain activity. Furthermore, our results suggest that the nature of the stimuli used in the study (trauma-related or unrelated) may also have an important impact upon resultant brain activity in PTSD relative to both control groups (trauma-exposed and trauma-naïve). We review findings in the context of extant neurobiological models. We propose important future directions for neuroimaging work in this area, and also explore the clinical relevance for trauma-exposed individuals who do not develop full-blown PTSD.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

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Program#/Poster#: 427.03/BB1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: VA Merit grant (TDG)

VA Merit grant (RS)

Title: Concurrent measurement of Neuropeptide Y (NPY) in cerebrospinal fluid (CSF), plasma, and saliva from combat veterans with and without posttraumatic stress disorder (PTSD)

Authors: *R. SAH[1], N. N. EKHATOR[2], L. JEFFERSON-WILSON[2], P. HORN[3], T. D. GERACIOTI[2]
[1]Psychiatry & Behavioral Neurosci., Univ. Cincinnati, CINCINNATI, OH; [2]Veterans Affairs Med. Ctr., Cincinnati, OH; [3]Mathematical Sci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Neuropeptide Y (NPY), a 36-amino acid peptide transmitter, is recognized as a resilience-to-stress factor in humans. Previous work from our group found reduced cerebrospinal fluid (CSF) concentrations of NPY in veterans with combat-related PTSD in comparison with both healthy volunteers and combat veterans without PTSD. Studies on plasma NPY in combat PTSD subjects from other groups have observed both, reduced or increased levels that are dependent on trauma exposure or recovery, respectively. In the current study we performed serial measurement of NPY in CSF, plasma, and saliva samples collected from combat veterans with or without PTSD over a 6 hour time interval. The objective was, a) to extend previous single time

point studies to continuous sampling in order to examine repeated measures data, including afternoon CSF NPY levels, b) to compare simultaneously-obtained plasma and saliva pools with CSF to explore their potential as biomarkers of central NPY. Consistent with our previous findings with a single time point collection, we observed an enduring reduction in CSF NPY in veterans with PTSD as compared with the combat-no PTSD group. Two way ANOVA, using diagnosis and time as variables, revealed a statistically significant effect of diagnosis [$F_{(1,111)} = 7.80$; $p=0.006$] with no time or diagnosis x time interaction. No significant difference in plasma or saliva NPY concentrations between the combat-PTSD and combat-no PTSD cohorts was observed. Collectively, our data indicate that decreased CSF NPY concentrations in combat veterans with PTSD appear to be a feature of the disorder, and one that persists throughout the day (ruling out acute stress-related responses during lumbar puncture). Additionally, plasma and salivary pools did not reflect CNS NPY alterations in PTSD, at least under baseline conditions of the current study. Funded by VA merit grants (TDG, RS)

Disclosures: R. Sah: None. **N.N. Ekhator:** None. **L. Jefferson-Wilson:** None. **P. Horn:** None. **T.D. Geracioti:** None.

Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH 5RO1MH054636

NDSEG Fellowship to MBV

VET Registry

Title: Behavioral and brain responses to ambiguous facial expressions in posttraumatic stress disorder

Authors: *L. M. SHIN[1,2], M. B. VANELZAKKER[1,2], L. K. STAPLES-BRADLEY[1,3], S. DUBOIS[1], K. C. HUGHES[2], N. B. LASKO[2], M. K. DAHLGREN[1,2], P. PANIC[1], R. OFFRINGA[1], B. I. HAKIM[1], N. J. CARTER[1], F. C. DAVIS[2,4], E. T. WHITE[1], L. M. LAIFER[1], R. E. KORUS[1], T. H. CHAN[1], N. M. SHEN[1], S. P. ORR[2], R. K. PITMAN[2]

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Abstract: Previous research has shown that posttraumatic stress disorder (PTSD) is associated with exaggerated amygdala activation to clear indicators of potential threat in the environment (i.e., fearful facial expressions; e.g., Rauch et al, 2000; Shin et al., 2005; Williams et al., 2005). However, many individuals with PTSD are hypervigilant when threat is not clearly present. In the current research, we sought to study amygdala responses to ambiguous social signals (i.e., surprised facial expressions, which can be interpreted positively or negatively [Kim et al., 2003]) in PTSD. Using functional magnetic resonance imaging (fMRI) and a well-validated set of surprised facial expressions (Ekman & Friesen, 1976), we studied Vietnam combat veterans with PTSD (n=12) and without PTSD (n=16). The two groups did not significantly differ in their categorization or valence ratings of the surprised faces, but the PTSD group demonstrated greater amygdala activation to the surprised expressions than the non-PTSD group. Thus, although there appeared to be no interpretation bias on the behavioral level, greater amygdala activation in the PTSD group could reflect greater evaluation of the ambiguous social stimuli.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.05/BB3

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Dutch Ministry of Defense

Title: Neuropeptide Y and the association with deployment to a combat zone and symptoms of post traumatic stress disorder: A longitudinal prospective military cohort study

Authors: *E. GEUZE[1,2], A. REIJNEN[1,2], E. VERMETTEN[1,3,2,4]
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Care, Utrecht, Netherlands; [3]Leiden Univ. Med. Ctr., Leiden, Netherlands; [4]Arq Psychotrauma Expert Group, Diemen, Netherlands

Abstract: Introduction: Neuropeptide Y (NPY) is a peptide neurotransmitter highly expressed in the limbic and brain stem areas and in the autonomic nervous system. NPY is suggested to represent a biological correlate of resilience, promoting stress regulation. Therefore, it is relevant to examine NPY in studies on the neurobiology of stress-related disorders, such as posttraumatic stress disorder (PTSD). Reduced NPY concentrations are found in PTSD patients compared to healthy controls. However, studies have also reported reduced baseline NPY levels to be associated with trauma exposure rather than PTSD. The aim of the current study is to assess the effect prolonged high-intensity stress on plasma NPY levels and the association with the development of PTSD symptoms. Method: This study is part of a prospective cohort study on deployment-related health problems in the Dutch armed forces. A total of 920 males volunteered to participate in the study prior to a 4-month deployment to Afghanistan. About one month prior to deployment and at 1 and 6 months post-deployment participants completed various questionnaires and blood samples were collected. In addition, follow-up assessments with questionnaires were performed at one and two years after return. The presence of PTSD symptoms was assessed with The Dutch Self-Rating Inventory for PTSD (SRIP). Results: Preliminary findings show a significant increase in plasma NPY levels shortly after deployment. Further analyses will be performed to test the hypothesis that NPY levels are lowered in participants with a high level of PTSD symptoms and to test if these NPY levels are low pre-trauma or if this is evoked by trauma. Discussion: Plasma NPY levels increase after deployment to a combat-zone. The (alterations in) NPY levels might be associated with the development of PTSD symptoms. More insight on NPY as a vulnerability and/or protective factor might provide treatment options for military personnel with a high level of PTSD symptoms, and might also provide potential preventive interventions strategies.

Disclosures: **E. Geuze:** None. **A. Reijnen:** None. **E. Vermetten:** None.

Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.06/BB4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: DoD W81XWH-10-1-0925

Title: Frontal cortex activity during emotional processing is altered in veterans with hazardous alcohol use and posttraumatic stress disorder

Authors: G. FORSTER[1], *D. M. OLSON[1], L. BAUGH[1], J. HANSEN[2], S. ENGEL[1], R. GAHER[2], J. SIMONS[2], V. MAGNOTTA[3]
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Abstract: Emotionally-significant stimuli activate frontal cortical regions such as the anterior cingulate cortex (ACC), 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 the ACC. 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. However, other studies show increased activation of the ACC with combat-related imagery in veterans with PTSD. 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. 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 hazardous 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 hazardous alcohol use. Eighty-five male and female right-handed veterans of Operations Enduring Freedom and Iraqi Freedom (OEF/OIF) were assessed for combat intensity, PTSD, alcohol use and alcohol dependence, and completed the Wisconsin Card Sorting Task (WCST) as a measure of executive functioning. Participants underwent functional magnetic resonance imaging (fMRI) 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. However, there was no effect of PTSD on any measure on the WCST, suggesting that frontal-related behavioral alterations might be specific to emotional processing in veterans with PTSD. Interestingly, veterans with PTSD and hazardous alcohol use showed increased activity of the rostral ACC in combat word conditions of the ECST as compared to controls (veterans without PTSD or hazardous alcohol use); a pattern not observed in PTSD or hazardous alcohol groups alone. Overall, findings suggest that hazardous alcohol use might contribute to inconsistencies in rostral ACC reactivity between different imaging studies of PTSD populations.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.07/BB5

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Institute for Clinical Research, Inc.

War-Related Illness and Injury Study Center, Washington, DC

Title: Antisaccade as an objective measure of inhibitory control in PTSD: an eye-tracking study

Authors: M. J. REINHARD[1], L. M. WONG[1], S. BANSAL[2], N. ALLEN[1], *B. L. SCHWARTZ[1]
[1]VA Med. Ctr., Washington, DC; [2]George Mason Univ., Washington, DC

Abstract: BACKGROUND: The antisaccade task is used to assess the competition between involuntary, stimulus-driven (bottom-up) and voluntary (top-down) eye movements and attention. Neuroimaging, animal studies, and single-cell recording have verified the central role of inhibitory brain mechanisms in antisaccade performance. Because inhibitory control difficulty is a salient feature of PTSD, we assessed whether antisaccade performance was impaired in this population, and whether performance related to a clinical neuropsychological measure of attention and inhibitory control METHOD: Veterans with (PTSD+; n=10) and without PTSD (PTSD-; n=10) were administered an eye movement task, with fixation on a center point and a target circle presented peripherally. They were asked to look toward the target (prosaccade) or in the direction opposite the target (antisaccade). Errors for both tasks were compared between groups, and correlated to PTSD symptom scores and the continuous performance test (CPT). RESULTS: There was no difference between groups on error rate for the prosaccade task ($t = -1.14$, $p = 0.27$), however the PTSD+ group made significantly more errors than the PTSD- group on the antisaccade task ($t = -2.30$, $p = 0.04$). In PTSD+, increased errors in the prosaccade task were associated with increased avoidance and numbing symptoms ($r = 0.63$, $p = 0.05$), and in the antisaccade task were associated with increased re-experiencing symptoms ($r = 0.74$, $p = 0.01$). Groups did not differ in performance on the CPT. In PTSD+, increased errors in the antisaccade task were associated with increased commission errors ($r = 0.88$, $p = 0.002$) and decreased d-prime ($r = -0.87$, $p = 0.002$) in the CPT. In PTSD-, these associations were not observed. CONCLUSIONS: This study demonstrates that antisaccade performance: (1) differs between PTSD+ and PTSD- veterans, (2) declines with increased PTSD symptoms, and (3) relates to inhibitory control impairment in a clinical neuropsychological test. Findings suggest that the

antisaccade task is a sensitive and potentially reliable neurobehavioral marker of inhibitory control disturbances in PTSD.

Disclosures: **M.J. Reinhard:** None. **L.M. Wong:** None. **S. Bansal:** None. **N. Allen:** None. **B.L. Schwartz:** None.

Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.08/BB6

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Institute for Clinical Research, Inc.

Title: More than meets the eyes: the impact of eye gaze and emotional expression on antisaccade performance in PTSD

Authors: ***L. M. WONG**[1], S. BANSAL[2], N. ALLEN[1], A. H. ADAMS[1], B. L. SCHWARTZ[1], M. J. REINHARD[1]
[1]VA Med. Ctr., Washington, DC; [2]George Mason Univ., Washington, DC

Abstract: BACKGROUND: Social avoidance and interpersonal difficulty are core symptoms of PTSD, and inhibitory control impairments are often observed in individuals with the disorder. In many social interactions, one must interpret human directional gaze cues and emotional expressions, and respond accordingly−looking in the same direction of someone's gaze is an automatic response. The antisaccade task has been used in many populations to assess inhibitory control, the ability to suppress an automatic response. We examined how directional gaze and affect impact inhibitory control in PTSD, and whether performance related to a clinical neuropsychological measure of executive function. METHOD: Participants were veterans with PTSD (PTSD+; n=11) or without PTSD (PTSD-; n=10). They viewed centrally presented neutral and fearful faces, the eye gaze of which was either to the left or right. In the Prosaccade and Antisaccade tasks, respectively, participants looked in the same or opposite direction of the eye gaze. Dependent variables were error rate and first accurate saccade latency. These variables were compared between groups, and correlated to the letter-number sequencing task. RESULTS: In a Group (PTSD+ and PTSD-) X Task (Prosaccade and Antisaccade) X Emotion (neutral and fear) ANOVA, there was no effect of Group on errors ($F = 0.05$, $p = 0.83$), though people in both groups do make more errors in the Antisaccade task ($F = 0.72$, $p = 0.03$). There was an effect of

Group on latency, such that PTSD+ was slower overall ($F = 6.22$, $p = 0.01$), which was driven by slowing in the Antisaccade ($t = -2.22$, $p = 0.04$) but not Prosaccade task ($t = -0.33$, $p = 0.74$). Inhibitory cost was calculated as the increase in first accurate saccade latency in the Antisaccade relative to Prosaccade task. Inhibitory cost did not differ between groups ($t = -0.94$, $p = 0.36$), but in PTSD+ was associated with impaired performance on a letter-number sequencing task ($r = -0.68$, $p = 0.04$). CONCLUSIONS: This study demonstrates that human directional gaze (1) can be used in a social version of the antisaccade task, (2) is sensitive to inhibitory control impairment in PTSD, and (3) can be used in a task that relates to other measures of executive function. These findings suggest that eye-tracking measures are an effective tool in examining how social and affective cues affect cognitive function in PTSD.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.09/BB7

Topic: E.05. Stress and the Brain

Support: US Army Grant DM102881

Title: Antagonism of melanocortin 4 receptors by intranasal HS014 attenuates single prolonged stress triggered changes in several brain regions

Authors: *L. I. SEROVA[1], M. LAUKOVA[2], L. G. ALALUF[1], E. L. SABBAN[1]
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Abstract: The melanocortin receptor four (MC4R) is expressed in many brain regions and is implicated in regulation of stress-related functions. We have previously shown that intranasal (IN) infusion of MC4R antagonist HS014, shortly before single prolonged stress (SPS) animal model of PTSD can attenuate development of several behavioral impairments. Male Sprague Dawley rats infused IN with 100 μg HS014 not only had reduced despair behavior in forced swim immediately following immobilization stress but also attenuated development of depressive- and anxiety-like behavior induced by traumatic stress of SPS. However, low dose HS014 (3.5 ng) effectively attenuated only anxiety-like behavior (Serova et al. 2013, Beh. Brain

Res,.250:139-147). Here we evaluated their effects on SPS-elicited changes in HPA axis and expression of genes of interest in mediobasal hypothalamus, hippocampus and locus coeruleus. Rats were given IN HS014 (3.5 ng or 100 µg) or vehicle 30 min prior to SPS stressors or left unstressed (controls). Acute responses were evaluated 30 min following SPS stressors. HS014 infusion led to smaller rise in plasma corticosterone (100 µg HS014) as well as absence of induction of corticotropin-releasing hormone (CRH) mRNA in mediolbasal hypothalamus (both doses) and of mRNA for norepinephrine biosynthetic enzymes (TH and DBH) in locus coeruleus (especially 3.5 ng HS014). Long-term responses were examined 7 days after SPS stressors, time requested for developing PTSD-related symptomology. In the mediobasal hypothalamus, SPS triggered induction of CRH mRNA which was attenuated with HS014 (both doses). However, HS014 was not effective to change the SPS-elicited elevation in mRNAs for the glucocorticoid receptor (GR) and FKBP5, a component of GR co-chaperone complex. In the ventral hippocampus, the SPS-elicited increase of GR protein levels was absent in animals given HS014 (both doses). The results demonstrated that antagonism of MC4R by intranasal infusion prior to traumatic stress attenuates development of molecular abnormalities in several brain regions implicated in mediating PTSD-associated impairments.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 427.10/BB8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: ZonMw grant 91210041

Title: Intranasal oxytocin affects amygdala reactivity towards emotional faces in recently traumatized individuals

Authors: *J. FRIJLING[1], M. VAN ZUIDEN[2], S. B. J. KOCH[2], L. NAWIJN[2], D. J. VELTMAN[3], M. OLFF[2,4]
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Abstract: Background: Few interventions are available that effectively prevent post-traumatic stress disorder (PTSD) development early post-trauma. PTSD is characterized by amygdala hyperresponsiveness, which also may be a pre-existing risk factor for PTSD development. Intranasal oxytocin (OT) may prevent PTSD early after trauma, since it targets amygdala reactivity. Here we used fMRI to study the effects of intranasal OT on amygdala reactivity in recently traumatized individuals at increased PTSD risk. Since effects of OT may differ between males and females, we additionally aimed to explore differential effects of sex. Methods: In a randomized double-blind placebo-controlled fMRI study we assessed amygdala responses towards emotional faces (fearful, happy, neutral) in 41 recently trauma-exposed individuals (M = 17). Participants were recruited from Emergency Departments after experiencing a traumatic event. Within 7 days post-trauma trauma-exposed individuals were assessed for increased PTSD risk using 2 questionnaires. Within 11 days post-trauma participants were scanned after either intranasal OT (40 IU) or placebo administration. Treatment effects were assessed for each emotion separately. Small volume corrected analyses were conducted for bilateral amygdala. Post hoc, we tested for differential effects of sex. Results: Compared to placebo, OT administration increased right amygdala (xyz 29 3 -21) reactivity during processing of fearful faces. Exploratory analyses showed that OT increased left amygdala (xyz -26 -9 -15) reactivity during processing of neutral faces in females, and also tended to decrease right amygdala (xyz 25 -11 -11) reactivity during processing of fearful faces in males. Conclusion: These results indicate that OT affects amygdala reactivity in recently traumatized individuals, however the effects depend on stimulus valence and sex. The exploratory analyses revealed a possible dual effect of OT in the right amygdala during fearful face processing in males. The influence of sex and other interindividual factors (e.g. acute PTSD symptom severity) on the effects of OT on the amygdala should be further investigated.

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Poster

427. Post-Traumatic Stress Disorder and Brain Trauma

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Program#/Poster#: 427.11/BB9

Topic: E.05. Stress and the Brain

Support: NIMH (R01 MH084966)

U.S. Army Research Office and Defense Advanced Research Projects Agency (Grant W911NF-10-1-0059)

Title: Persistently elevated ghrelin underlies continued vulnerability to enhanced fear following stress exposure

Authors: *E. S. HARMATZ, K. GOOSENS
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Abstract: In humans, prolonged stress exposure produces vulnerability to trauma-associated mental health disorders such as post-traumatic stress disorder (PTSD) for years after stressor exposure is terminated. We have recently shown that chronic stress produces vulnerability to enhanced fear in a rat model of PTSD by elevated signaling through ghrelin and growth hormone. Specifically, chronic stress persistently enhances circulating peripheral levels of acylated ghrelin and also levels of growth hormone in the basolateral amygdala. We hypothesized that the persistent elevation in circulating ghrelin might underlie the long-term vulnerability to PTSD that follows stress exposure. To address this issue, we first examined circulating ghrelin levels in rats seventy days following stressor termination. Rats were exposed to immobilization stress (STR) or handling (no stress, or NS) for two weeks and tail blood samples were collected seventy days following the final stress or handling session. We found that ghrelin levels were significantly elevated even at this remote time point. Because we have previously shown that antagonism of the ghrelin receptor during stress exposure prevents stress-related enhancement of fear, we conducted an experiment to determine whether stress-enhancement of fear would re-emerge if ghrelin receptor antagonism was terminated at the end of stress exposure. Rats were exposed to 14 days of either STR or NS and received injections of either vehicle (VEH) or the ghrelin receptor antagonist GHRP-6 [D-Lys3] (DLys) daily; all rats received vehicle injections for 14 days after the final stress or handling session. Tail blood samples were collected during the post-stress period to determine whether DLys administration affected endogenous circulating ghrelin levels. Auditory fear conditioning was administered on the fifteenth day following the final stress or handling session. This resulted in the following groups: NS-Veh/Veh, STR-DLys/Veh, STR-Veh/Veh. We found that rats in both the STR-DLys/Veh and STR-Veh/Veh groups displayed enhanced fear memory relative to rats in the NS-Veh/Veh group. Moreover, the administration of DLys during stress did not prevent stress-associated enhancement of circulating ghrelin. These studies are consistent with the idea that stress-related enhancement of ghrelin contributes to continued vulnerability to fear after stress exposure. **Keywords:** Stress, Amygdala, Learning, Restraint, PTSD, Rat

Disclosures: E.S. Harmatz: None. **K. Goosens:** None.

Poster

428. Alcohol: Intake and Preference

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 428.01/BB10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA023281

NIH grant AA014351

Title: N/OFQ system selective antagonism decreases binge-like alcohol drinking in mice

Authors: *G. BRUNORI[1,2], A. CIPPITELLI[1], A. OZAWA[1], J. SCHOCH[1], M. GORMAN[1], K. GAIOLINI[1], N. ZAVERI[3], R. CICCOCIOPPO[2], L. TOLL[1]
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Abstract: Several lines of evidence suggest a role of the N/OFQ system in alcohol-related behaviors. The 'drinking in the dark' (DID) procedure is a well-established animal model of human binge-like alcohol drinking known to reliably generate high levels of voluntary alcohol consumption and pharmacologically relevant blood alcohol concentration in alcohol preferring mice strains (e.g. = C57BL/6J). Frequent binge drinking facilitates alcohol dependence. Thus, the DID model may represent a useful tool for studying the neurobiology underlying the transition to dependence. In the present study the contribution of N/OFQ receptor (NOP) signaling in the binge-like drinking behavior is investigated. During the 4-day DID procedure C57BL/6J mice received alcohol (20% v/v) in place of water, beginning 3 hours into the dark cycle. Mice had 2 hours access to alcohol solution during days 1 to 3, and 4 hours on day 4. On the fourth day, mice were given a subcutaneous injection of the NOP selective agonist AT 202 (0, 3, 10 mg/kg) or antagonist SB 612111 (0, 3, 10, 30, 60 mg/kg) 30 minutes before receiving alcohol. We have determined that binge-like alcohol drinking was significantly attenuated by pretreatment with SB 612111 (30, 60 mg/kg), while pretreatment with AT 202 did not alter the consumption. The finding that the NOP antagonist SB612111 can attenuate alcohol drinking indicates that NOP receptors are involved in excessive alcohol intake before the development of dependence. Current studies are aimed at evaluating the alterations of the N/OFQ system signaling using immunoreactivity mapping of the NOP receptor on NOPeGFP knock-in mice (generated on the C57BL/6J-mouse background).

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant AA020929

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Title: Both forced swim stress and kappa opioid receptor activation accelerate escalation of dependence-related ethanol consumption in C57BL/6J mice

Authors: *R. I. ANDERSON[1], C. D. PHELPS[3], I. T. ROBBINS[3], M. F. LOPEZ[2], H. C. BECKER[2]
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Abstract: Stress exposure may facilitate the transition from casual alcohol use to excessive drinking associated with dependence. Our lab has previously found that daily forced swim stress (FSS) prior to ethanol drinking sessions results in enhanced ethanol consumption in mice with a history of chronic intermittent ethanol (CIE) vapor exposure but not air-exposed controls (CTL). Kappa opioid receptors (KORs), which are activated by stress and upregulated by chronic ethanol, pose one potential mechanism by which stress may interact with ethanol dependence. Other laboratories have shown that administration of the KOR agonist U50,488 can mimic behavioral effects of FSS. Accordingly, the present study was designed to determine whether U50,488 administration could substitute for the effects of repeated daily FSS on ethanol consumption in CIE and CTL C57BL/6J mice. Once stable baseline ethanol intake was established in a 1-bottle (15% ethanol) limited access (1 hr/day) procedure, mice received chronic intermittent exposure (16 hr/day x 4 days/week) to ethanol vapor (CIE group) or air (CTL group). The weekly cycles of inhalation exposure were alternated with 5-day drinking test cycles. Intake sessions were conducted in drinking chambers configured with lickometer circuitry. CIE and CTL groups were further subdivided into stress conditions: 1) no stress, 2) FSS (10 min; 4 hr prior), or 3) 5 mg/kg injection of U50,488 (in lieu of FSS exposure) 60 min prior to each ethanol drinking test session. Blood samples were collected after the last drinking

session each week for BEC analysis. Analysis of lick data, g/kg intake, and BEC values revealed similar results: CIE mice exposed to FSS demonstrated escalated ethanol consumption after only one cycle of CIE exposure, an effect not seen in CTL mice or in CIE mice that did not experience stress. Administration of the KOR agonist U50,488 produced a similar pattern of increased ethanol consumption. These data support a role for KOR activity in contributing to stress-induced facilitation of ethanol dependence-related drinking. Future work will assess whether a KOR antagonist can block the effects of FSS on ethanol consumption in the dependence model. Supported by NIH grants AA020929, AA014095, AA010761, AA007474 and VA Medical Research. Keywords: ethanol, stress, dynorphin

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Oral operant ethanol self-administration in the absence of explicit cues, food restriction, water restriction or ethanol fading in C57BL/6J mice

Authors: A. M. STAFFORD, S. M. ANDERSON, K. L. SHELTON, *D. H. BRUNZELL
Dept. of Pharmacol., Virginia Commonwealth University, MCV, RICHMOND, VA

Abstract: The goal of these studies was to develop a mouse model of *oral operant* ethanol (ETOH) self-administration that did not involve explicit cues, food restriction, water restriction, or the fading of ETOH. This experimental design enabled assessment of initiation and escalation of ETOH reinforcement across doses (0, 3, 10 and 15% by volume). Separate cohorts of C57BL/6J male mice received ETOH in 0.2% saccharin (SAC) or water (H_20) vehicle reinforced on a variable ratio 5 (VR5) schedule across 9 weekly overnight sessions. To achieve equal levels

of responding upon initial ETOH exposure, all mice were first trained to lever press for 0.2% saccharin on an accumulating schedule of reinforcement to VR 5. Mice had access to food and water bottle during all ETOH self-administration sessions. ETOH self-administration in SAC revealed dose-dependent increases in ETOH consumption, but dose-associated decreases in number of reinforcers earned over sessions. Preference studies, comparing water bottle consumption to liquid dipper consumption, showed that mice reinforced with SAC ingested ~80% of their fluid from the liquid dipper independent of ETOH dose. Preference to work for fluid rather than drink from the available water bottle suggested that saccharin vehicle was rewarding and may have precluded the observation of ETOH reinforcement in the operant task. In contrast, ETOH self-administration using H_20 consistently revealed dose-associated increases in ETOH consumption and reinforcers earned; mice receiving 15% ETOH showed significant increases in ETOH consumed and reinforcers earned compared to H_20 vehicle mice, as well as an escalation in reinforcers earned over weeks. In contrast to mice in the SAC study, mice reinforced with H_20 vehicle drank ~20% of their fluid from the liquid dipper. Compared to vehicle subjects, mice receiving 15% ETOH in water showed significantly greater preference for consumption from the liquid dipper suggesting that this dose of ethanol was rewarding. To test if this model has predictive validity for therapeutic efficacy, subsequent operant testing following naltrexone injection resulted in a significant reduction of reinforcers earned and ETOH consumed in 15% ETOH mice without affecting behavior of vehicle and low dose ETOH mice. These results validate this novel mouse model of oral operant ETOH self-administration that, in the absence of sweetners to mask ETOH, ETOH fading, food/water deprivation and explicit cues, can be utilized to investigate biological, genetic, and pharmacological contributions to ETOH reinforcement.

Disclosures: A.M. Stafford: None. **D.H. Brunzell:** None. **S.M. Anderson:** None. **K.L. Shelton:** None.

Poster

428. Alcohol: Intake and Preference

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 428.04/BB13

Topic: C.17. Drugs of Abuse and Addiction

Support: Swedish Research Council, Sweden

Linköping University, Sweden

Title: A novel, peripherally available Nociceptin/Orphanin FQ receptor agonist SR-8993 reverses hangover anxiety, attenuates alcohol intake, and prevents reinstatement in Wistar rats

Authors: *A. M. AZIZ[1,2], S. BROTHERS[3], L. HOLM[2], M. HEILIG[4], C. WAHLESTEDT[3], A. THORSELL[2]
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Abstract: Aims: The present study aimed to examine the efficacy of a novel, blood-brain barrier penetrant Nociceptin/Orphanin FQ (NOP) receptor agonist, SR-8993, in rodent behavioral models of anxiety and alcohol-intake related behavior. The NOP receptor has been shown to play an important role in the regulation of reward and motivation pathways. Previous studies have demonstrated that NOP receptor activation by central administration of its endogenous ligand reduces alcohol intake, attenuates alcohol-induced conditioned place preference, and blocks stress-induced reinstatement of alcohol seeking in rats. **Methods:** Male Wistar rats were used in all experiments. SR-8993 was given intraperitoneally (ip) at a dose of 1 mg/kg. Anxiety-related behavior was measured using the elevated plus-maze (EPM), with or without a preceding acute administration of alcohol (3 g/kg; 12hrs prior to EPM) to model alcohol withdrawal-induced anxiety. Alcohol intake was examined in non-operant, two-bottle free-choice models, as well as operant self-administration. Operant responding was additionally used to measure motivation (progressive-ratio responding) and stress/cue-induced reinstatement of responding/alcohol seeking. Relapse following protracted abstinence for operant self-administration was examined following a three (3) week period of no alcohol access. **Results:** SR-8993 is mildly anxiolytic and reverses alcohol induced hangover anxiety-related behavior in the EPM. It reduces alcohol-intake and preference in both non-operant and operant intake paradigms. In addition, an attenuation of progressive-ratio responding was seen following NOP agonist treatment indicating decreased motivation to lever press for alcohol. With regards to reinstatement of alcohol-seeking, stress (yohimbine, ip, 1.25 mg/kg) and cue (orange odor) induced reinstatement of responding was blocked by pre-treatment with SR-8993. Protracted abstinence increased overall responding (the alcohol deprivation effect), and an increased sensitivity to SR-8993 was seen with a lower dose (0.33 mg/kg) suppressing lever-pressing for alcohol. **Conclusions:** These findings indicate that NOP agonism may be a putative effective treatment of anxiety- and alcohol-related states, and warrants further investigation.

Disclosures: A.M. Aziz: None. **S. Brothers:** None. **L. Holm:** None. **M. Heilig:** None. **C. Wahlestedt:** None. **A. Thorsell:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Swedish Research Council

Alcohol research council of the Swedish alcohol retailing monopoly

Jeansons foundation

Åke Wiberg foundation

Swedish Society of Medicine

Title: A glucagon like peptide-1 analogue reduces alcohol intake and prevents relapse relapse drinking

Authors: *E. JERLHAG[1], E. EGECIOGLU[2], J. A. ENGEL[3]
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Abstract: Alcohol dependence is a heterogeneous disorder where several signaling systems play important roles. By understanding the complex mechanisms underlying this disease new treatment strategies may be developed. While the gut-brain hormone glucagon like peptide-1 (GLP-1) was gaining status as food and glucose regulating peptides, the work pinpointing the midbrain dopamine system as a target GLP-1, led us to hypothesize that this hormone may have a role that extends beyond regulation of food intake to include reward seeking behavior, such as reward induced by and intake of alcohol. Therefore, the effect of a GLP-1 analogue (liraglutide) on alcohol intake and relapse to alcohol drinking following abstinence was investigated in rats. We have shown that acute administration of liraglutide reduces alcohol consumption in rats exposed to alcohol for ten weeks as well as prevents relapse drinking in rats using the alcohol deprivation model. Moreover, repeated administration of liraglutide reduces alcohol intake in rats. Supportively, previously we showed that another GLP-1 analogue (exendin-4) suppresses the alcohol-, amphetamine, cocaine- and nicotine-induced reward as well as reduces voluntary alcohol consumption and alcohol seeking behaviors in rats. This implies that gut-brain hormones, such as GLP-1 may be involved in reward regulation. Collectively, our data indicate that GLP-1 receptors may constitute a novel potential target for treatment of drug dependence.

Disclosures: **E. Jerlhag:** None. **E. Egecioglu:** None. **J.A. Engel:** F. Consulting Fees (e.g., advisory boards); Pfizer AB Sweden, Lundbeck AB Sweden, Actavis AB Sweden.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Department of Anesthesiology RAP Grant

Title: Ethanol drinking is potentiated in mice expressing a "humanized" A118G mu opioid receptor polymorphism

Authors: A. N. HENDERSON-REDMOND[1], T. S. LOWE[1,3], X. B. TIAN[1], C. M. NEALON[1], *D. J. MORGAN[1,2]
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Abstract: The mu-opioid receptor (MOR) has been implicated in mediating the rewarding effects of various drugs of abuse, including ethanol. In human populations, the single-nucleotide polymorphism (SNP) of the OPRM1 gene with the greatest association with drug addiction potential is the A118G allele. Some clinical studies suggest that 118G allele might increase reward for ethanol and responsiveness to naltrexone. Further, "humanized" mice homozygous for the 118GG allele showed a 4-fold greater release of dopamine in the nucleus accumbens following an ethanol challenge relative to 118AA mice. Taken together, these results suggest that the 118G allele may confer a genetic vulnerability to ethanol addiction and reward. Therefore, the purpose of the present study was to assess whether the A118G polymorphism potentiated consumption and reward for ethanol. Mice homozygous for the "humanized" 118AA or 118GG alleles were evaluated to test the hypothesis that ethanol consumption, ethanol reward, and responsiveness to naltrexone is increased in 118GG mutants. The rewarding effects of ethanol were assessed using a conditioned place test (CPP) and ethanol intake was measured in both 24-hour continuous access and limited (Drinking-in-the-Dark; DID) access drinking paradigms. Sensitivity to the suppressive effects of naltrexone, an opioid receptor antagonist, were assessed in the DID limited access drinking paradigm. Our results revealed that 118GG females drank significantly more ethanol than littermate 118AA females in both the continuous (at 12% and 15% ethanol by volume) and limited access (DID) paradigms. Pretreatment with 1 mg/kg naltrexone significantly decreased ethanol intake in both 118GG and 118AA female mice in the DID paradigm suggesting that 118GG female mice are not more sensitive to naltrexone. Sucrose and quinine intake did not differ as a function of genotype suggesting that 118GG females possess normal taste sensitivity relative to control mice expressing the 118AA allele. Finally,

118GG but not 118AA female mice showed statistically significant conditioned place preference for ethanol raising the possibility that the 118GG allele might enhance the rewarding effects of ethanol. These findings indicate that 118GG female mice display increased consumption and reward for ethanol.

Disclosures: A.N. Henderson-Redmond: None. **T.S. Lowe:** None. **X.B. Tian:** None. **C.M. Nealon:** None. **D.J. Morgan:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: CONACYT scholarship 257934

PIFI scholarship 20101087

Title: Orexin A increases the alcohol dinking behavior of rats that drank alcohol in their childhood

Authors: *L. G. MENDOZA RUIZ[1,2], P. VAZQUEZ-LEÓN[2], L. MARTÍNEZ-MOTA[3], A. MIRANDA-PÁEZ[2]
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Abstract: Alcoholism is a relapsing chronic disorder characterized by compulsive alcohol drinking and the loss of the control of the intake. Adolescents between the ages 12 and 17 years old who drink alcohol, increase the likelihood of becoming alcoholic in their adulthood from 35.6% to 42.9%. A very important effect of alcohol is observed in the central nervous system in the reward circuit. Mechanism of action of alcohol includes binding with the GABAA receptor activating it and with the NMDA receptor inhibiting it. Alcohol also modifies the reward circuit increasing the levels of dopamine in the nucleus accumbens (NAc) and the prefrontal cortex (PFC). The orexins have also been involved in drug seeking behavior and relapse. The orexinergic system is part of the reward circuit in the brain and is constituted by orexins A and B (OxA y OxB) and their receptors type 1 and type 2 (OX1R y OX2R), which project widely from the lateral hypothalamus through the central nervous system. In the present study, male Wistar

rats (N= 42) were forced to drank ethyl alcohol (10% v/v) as the only liquid in their diet from postnatal (PN) day 21 to 67. Their voluntary alcohol intake was assessed until day 95 postnatal, followed by the first withdrawal period; such schedule of alcohol intake and withdrawal was repeated 3 times across the experiment. Locomotor activity was assessed through open field test. Stereotaxic surgery was performed to implant a cannula aimed to the right lateral ventricle. Orexin A was microinjected through the cannula and voluntary alcohol intake and locomotor activity was measured again after orexin A microinjection. Several alcohol expositions since the PN day 21 followed by periods of abstinence increase the preference of alcohol intake in the adulthood. The dose of 1 nmol/ul of orexin A microinjected into the lateral ventricle do not modify the water intake in rats, but increase their alcohol consumption when alcohol is available early in life; also, the amount of alcohol ingested is even higher in the rats that were exposed to alcohol since 21 day old. It was observed that locomotor activity increases in rats during abstinence periods. Orexin A also increases locomotor activity in all the rats, regardless of the amount of alcohol drunk during childhood or lack thereof. The age at which rats start to drink and the repeated periods with alcohol availability are critical to change their drinking behavior in their adult life. The main effect of the orexin A on open field exploratory activity in the current study is prominent in alcohol-abstinence periods, and it has been suggested that the craving for drugs of abuse including alcohol, is result mainly of the increased orexinergic system activity.

Disclosures: L.G. Mendoza Ruiz: None. **P. Vazquez-León:** None. **L. Martínez-Mota:** None. **A. Miranda-Páez:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Intramural Research Program

Title: Behavioral and neural effects of chronic ethanol exposure in adolescent mice

Authors: *N. J. JURY, H. C. BERGSTROM, A. HOLMES
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Abstract: The current literature describing responses of rodents to chronic ethanol exposure is biased towards studies that use male, adult subjects. This bias persists despite epidemiological data showing high rates of ethanol abuse in women and, increasingly, during adolescence. Furthermore, there is emerging evidence of increased susceptibility to the lasting neural effects of alcohol abuse in young people and women. Therefore, the goal of the current study was to compare male and female adolescent and adult C57BL/6J mice for the effects of chronic intermittent ethanol exposure (CIE). Mice were exposed to CIE through vapor inhalation over 4 weeks (beginning at either 4 or 8 weeks of age) and then tested (all as adults) for ethanol tolerance (loss of righting reflex), ethanol consumption (24-hour, 2-bottle drinking) and a set of rewarded operant behaviors that included measures of motivation (progressive ratio) and compulsive (footshocked) reward-seeking. In addition, the dendritic morphology of prefrontal cortical and amygdala pyramidal neurons was quantified by visualizing neurons in Thy1-GFAP mutant mice. Results showed that mice developed significant tolerance to the effects of acute ethanol (3-4 g/kg dose) challenge (loss of righting reflex) after CIE, irrespective of age or sex. Following CIE, blood ethanol concentrations measured after acute ethanol (3.5 g/kg) challenge were not different between groups, indicating there was no clear metabolic tolerance. Adult male mice showed significantly increased ethanol consumption (of a 15% ethanol solution) following CIE, as compared to air controls or pre-CIE baseline. By contrast, neither adolescent (regardless of sex) nor female mice exhibited elevated drinking in response to CIE, though the high levels of baseline drinking in these groups may have produced a 'ceiling effect' that prevented further increases due to CIE. Taken together, these results provide initial insight into the lasting behavioral effects of CIE as a function of age and sex, and could ultimately have implications for understanding how women and young people may be at increased risk for alcohol abuse.
Research was supported by the NIAAA Intramural Research Program.

Disclosures: **N.J. Jury:** None. **A. Holmes:** None. **H.C. Bergstrom:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Ithaca College-Center for Faculty Excellence

Title: The role of maternal separation and subordination behavior during defeat stress on alcohol intake and sensitivity to alcohol-related behaviors

Authors: *B. E. CALDWELL[1], E. JACOBS-BRICHFORD[2], K. F. FARAG[2]
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Abstract: The role of early life stress and adult subordination during defeat stress on alcohol intake and sensitivity to alcohol-related behaviors. The relationship between stress and risk for developing alcohol (ETOH) use disorders has suggested that individuals are at risk for developing maladaptive coping strategies if they are exposed to early life trauma or social stress. The present study examined the effects of maternal separation (MS) stress during the postnatal period, followed by social defeat stress (DS) in adulthood, on ETOH drinking and locomotor sensitization in male Long Evans rats. Litters were randomly assigned to either MS or animal facility rearing (AFR). Separation periods were 180 minutes per day, beginning on post-natal day 1 (PND1), and continued for 14 days. Rats were then assigned to either DS or non-stress (NS) 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. Behaviors during defeat trials were videotaped and scored submission-related behaviors (e.g., supine posture, immobility). Results showed that MS animals had higher intake of ETOH, regardless of defeat status. MS and DS animals showed distinct patterns of ETOH, with MS animals drinking higher levels of 5% ETOH and DS animals drinking higher levels of 10% ETOH. No interaction was found between MS and DS, but the highest intake of ETOH was consistently found in animals exposed to both stressors (MSDS group). Analyses of locomotor behaviors in the open field showed AFR rats demonstrated an increase in sensitivity to the locomotor-stimulating effects of ETOH from Time 1 to Time 2. This increase was not observed in MS animals. The amount of recorded subordinate behaviors was inversely correlated with amount of ETOH consumed. The inverse correlation in subordination to ETOH, along with the increased ETOH intake in MS animals suggests that more subordination could be a more adaptive response to aggressive encounters, and MS animals are deficient in their ability to submit and appease the aggressive male, leading to a more stressful experience and higher preference for ETOH.

Disclosures: B.E. Caldwell: None. **E. Jacobs-Brichford:** None. **K.F. Farag:** None.

Poster

428. Alcohol: Intake and Preference

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Support: NCRR 5P20RR024485-02

NIGMS 8 P20 GM103542-02

NIAAA AA020930

Title: Proteomic analysis of cysteine-containing proteins identifies a role for glutathione-S-transferase pi and S-glutathionylation in alcohol dependence and consumption

Authors: *J. D. UYS[1], A. E. PADULA[3], M. F. LOPEZ[3], W. C. GRIFFIN, III[3], T. ANCRUM[1], L. E. BALL[1], D. M. TOWNSEND[2], P. J. MULHOLLAND[3]
[1]Dept Cell and Mol. Pharmacol., [2]Dept Pharmaceut. and Biomed. Sci., Med. Univ. South Carolina, CHARLESTON, SC; [3]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Oxidative-nitrosative stress (ROS/RNS) is a contributing factor to neurodegeneration associated with heavy chronic ethanol consumption. Compelling recent evidence suggests that oxidative stress signaling induced by prolonged drinking not only contributes to cellular injury, but may also influences the motivational states that drive heavy ethanol consumption. Cysteine residues are under-represented in mammalian proteins, but play critical roles in protein folding, antioxidant defense, and redox signaling. ROS/RNS can modify reactive cysteine residues in proteins, which includes the redox-mediated posttranslational modification, S-glutathionylation. S-glutathionylation of cysteine residues can dynamically influence cellular functioning under pathological conditions and is catalyzed by glutathione-S-transferase Pi (GSTP). The purpose of this study was to determine how chronic intermittent ethanol (CIE) exposure of C57BL/6J mice alters expression of cysteine-containing proteins and if these proteins play a role in regulating ethanol drinking. Multiplex iodoacetyl Tandem Mass Tags (iodo-TMT) tagging technology and liquid chromatography tandem mass spectrometry (LC-MS/MS) were used to identify and quantify redox-sensitive cysteine-containing proteins in the nucleus accumbens core (NAcc) of ethanol-dependent mice. Biological annotation revealed that most of the 618 identified proteins are localized to the cytosolic compartment (66.4%) and have either catalytic activity (34.0%) or binding function (30.7%). Interestingly, quantitation demonstrated that the glutathione-S-transferase (GST) class of proteins was altered after CIE exposure. Western blot analysis confirmed that CIE exposure significantly increased GSTP expression. Furthermore, using a fluorescent thiol specific probe, CIE exposure resulted in an increase in cysteine thiol modifications in the NAcc. Examination of intake levels using the drinking-in-the-dark model

revealed that mice with a genetic depletion of GSTP consumed significantly more ethanol than their wildtype (WT) littermates. The enhanced intake in the GSTP knockout (KO) mice was evident during the 2 and 4 hr drinking sessions. Sucrose consumption or metabolism of ethanol (3 g/kg, ig) did not differ between WT and GSTP KO mice. Together, these data demonstrate that CIE exposure of C57BL/6J mice significantly alters cysteine-containing proteins in the NAcc and that genetic depletion of GSTP alters ethanol intake. Thus, quantitative analysis of the cysteine proteome in the NAcc identified a novel target that is regulated by ethanol dependence and can influence voluntary ethanol consumption.

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Finnish Foundation for Alcohol Studies

JDTic was a gift from Research Triangle Institute

Title: Accumbally dosed mu- and kappa-opioid receptor agonists and antagonists modify ethanol intake in AA rats

Authors: J. UHARI-VÄÄNÄNEN[1,2], M. AIRAVAARA[3], P. BÄCKSTRÖM[1], V. OINIO[1,2], A. RAASMAJA[2], P. PIEPPONEN[2], *K. KIIANMAA[1]
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Abstract: The alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) lines of rats have proven useful in exploring the neural basis of ethanol addiction. Strain-specific differences found in the effects of opioids and in opioidergic mechanisms suggest that the opioidergic system plays a central role in the control of ethanol intake. The aim of the present study was to address the role of accumbal mu- and kappa-opioid receptors in controlling ethanol intake using male AA rats trained to drink 10% (v/v) ethanol solution in an intermittent,

time-restricted (90 min every other day) access paradigm. The mu-opioid receptor antagonist CTOP (0, 0.3, 1, or 3 ug per side), the mu-opioid receptor agonist DAMGO (0, 0.03, 0.1, or 0.3 ug), the kappa-opioid receptor agonist U50488H (0, 0.3, 1, or 3 ug), and the kappa-opioid receptor antagonists JDTic (15 ug) and nor-binaltorphimine (3 ug) were injected bilaterally into the nucleus accumbens shell. All drugs were administered in a volume of 0.3 ul at the rate of 0.3 ul/min. To eliminate carry-over effects of short acting drugs (CTOP, DAMGO, U50488H), there were two drug-free ethanol intake sessions between doses. Because of the long-lasting effects JDTic and nor-binaltorphimine they were administered only once. In another experiment, rats received a single subcutaneous injection of JDTic (10 mg/kg) and the long-term effects of the treatment on ethanol intake were monitored. CTOP, DAMGO and U50488H failed to alter ethanol intake significantly, though CTOP (0.3 and 1 ug) tended to increase and DAMGO (0.1 ug) tended to decrease ethanol intake. There was a tendency for decreased ethanol intake 72h after accumbally dosed JDTic, while systemically dosed JDTic decreased ethanol intake at the 48h and 96h time-points. These results support the notion that central mu- and kappa-opioid receptors participate in controlling voluntary ethanol intake. The results also suggest that accumbal kappa-opioid receptors may have a more prominent role in ethanol intake than previously assumed.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: 1Z1A AA000466

Title: Effects of acute stress exposure on operant intravenous alcohol self-administration (iv-asa) in non-dependent drinkers

Authors: *B. L. STANGL[1], J. WESTMAN[1], M. ZAMETKIN[1], L. KWAKO[1], R. SINHA[2], V. A. RAMCHANDANI[1]
[1]NIAAA/NIH, Bethesda, MD; [2]Yale Univ., New Haven, CT

Abstract: The relationship between stress and alcohol use and problems has been well supported in dependent drinkers. However, the effects of stress on alcohol use and problems in non-dependent drinkers is less clear. The objective of this study was to characterize acute stress reactivity and relationship with operant IV alcohol self-administration (IV-ASA) in non-dependent drinkers, using a guided imagery script challenge. IV-ASA was assessed using the Computer-Assisted Self-infusion of Ethanol (CASE) method that allows individuals to self-administer alcohol while controlling the breath alcohol concentration (BrAC) using a physiologically-based pharmacokinetic (PBPK) model-based algorithm. Healthy non-dependent drinkers (N=10) completed three cue reactivity + IV-ASA sessions, each consisting of exposure to a 5-min audio recording of scripts based on personalized stories of stress, alcohol, and neutral-relaxing cues. Following assessment of cue-reactivity using subjective response (Subjective Units of Distress Scale, SUDS) and neuroendocrine (ACTH, cortisol) measures, participants were provided with the opportunity to self-administer IV alcohol using a free-access paradigm where they could press a button to receive short IV ethanol infusions. IV-ASA measures included average (AVG) and peak (PEAK) BrAC. Results demonstrated that the script challenge successfully induced reactivity, with significant increases in SUDS scores and greater increases in cortisol following the stress-cue script compared with neutral-cue scripts. Participants also showed higher IV-ASA, indicated by significantly higher AVG and PEAK BrACs following the stress-cue script in comparison to the neutral-cue script. These results indicate that non-dependent drinkers are susceptible to the effects of acute stress cues and demonstrate increased alcohol self-administration after exposure to stress-cues. These data may provide important information on the relationship between stress and alcohol seeking behavior that may underlie risk for alcohol-related problems.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA015687

Title: Extended access to a highly palatable cafeteria-style diet reduces VTA dopamine neuron activity and ethanol consumption

Authors: *J. B. COOK, L. M. HENDRICKSON, H. B. THAKKAR, H. MORIKAWA
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Abstract: The hedonic value of food is mediated, at least in part, by the mesostriatal dopamine system originating in the ventral tegmental area (VTA). Previous studies suggest that prolonged consumption of energy dense "highly palatable" foods can lead to addiction-like, compulsive food intake that is associated with blunting of the dopaminergic system. In rats, access to a highly palatable diet has been shown to reduce dopamine transmission and expression of postsynaptic dopamine receptors in the striatum. Chronic administration of drugs of abuse, such as cocaine and ethanol, produce similar deficits in mesostriatal dopamine signaling. This has lead to the idea that a hypodopaminergic state may be involved in sustaining both compulsive food and drug consumption. Interestingly, prior access to a highly palatable diet has been shown to reduce acquisition of cocaine self-administration. However, it is not known how prior access to a highly palatable diet affects ethanol consumption or VTA dopamine neuron physiology. In the present study, we first administered a highly palatable cafeteria-style diet consisting of bacon, potato chips, cheesecake, cookies, breakfast cereal, and chocolate candies to male Wistar rats for 3-4 weeks and measured basal dopamine neuron firing as well as D2 receptor-mediated autoinhibition in VTA brain slices. Cafeteria diet access produced an obese phenotype, significantly increasing body weight ($p < 0.001$) compared to the chow only group. We showed that basal dopamine neuron firing frequency was lower in the cafeteria diet group compared to rats that received chow only ($p < 0.01$). Furthermore, the amplitude of the inhibitory outward current produced by low concentration of D2 agonist quinpirole (100 nM) was increased in the cafeteria diet group ($p < 0.05$). To determine the effects of cafeteria diet access on ethanol consumption, male Wistar rats were given access to an ethanol solution (10% v/v, 2 hr/day) for a 10 day baseline period, followed by 4 weeks of cafeteria diet access. Immediately after the cafeteria diet phase all rats were again allowed to drink ethanol in a daily 2 hr limited access session. Prior cafeteria diet access reduced ethanol consumption over 3 weeks of testing ($p < 0.05$) compared to animals that received chow only. Overall, the results suggest that access to a cafeteria-style diet produces a hypodopaminergic state and reduces ethanol consumption for up to 3 weeks.

Disclosures: J.B. Cook: None. **L.M. Hendrickson:** None. **H.B. Thakkar:** None. **H. Morikawa:** None.

Poster

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Location: Halls A-C

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Program#/Poster#: 428.14/BB23

Topic: C.17. Drugs of Abuse and Addiction

Title: Alcohol-preferring P rats do not exert higher compulsive drinking despite increased motivation to consume alcohol

Authors: R. S. DULMAN, E. AUGIER, *H. SUN, M. HEILIG
Mol. Pathophysiology, NIH/NIAAA, BETHESDA, MD

Abstract: Abstract: While drug addictions afflict a minority of human drug users, nearly all rats successfully acquire self-administration, a traditional rodent model of drug abuse. Recently, Ahmed and colleagues have demonstrated a modified self-administration paradigm that offers repeated choices between a drug and a food or sugar alternative. This new choice model allows for division of rats into groups that are indifferent, prefer drug, or prefer sugar and only a minority of rats prefer drug, a subset that aligns with human addiction rates. This choice model may be a screen for compulsive drug use and has been generalized to alcohol drinking. 48 Wistar rats and 16 inbred Alcohol-preferring P rats were trained to self-administer 20% ethanol (EtOH) and drinking behavior was assessed on a fixed-ratio-2 reinforcement schedule. P rats consume significantly more 20% EtOH at baseline and have higher progressive ratio breakpoints. Rats were then trained in a mutually exclusive discrete choice procedure to choose between 20% EtOH and 0.2% saccharin, a non-caloric sweetener. After stabilization in the choice test, only minorities of P rats and Wistar rats prefer alcohol to saccharin. These proportions were not different between the two strains (19% for P rats, 17% for Wistar rats). These results indicate that high consumption does not necessarily signify compulsion or addiction and question the use of P rats as an animal model of alcoholism. P rats may however remain a good model for high alcohol consumption for studies regarding EtOH-induced neurotoxicity. More generally, using the choice model to screen for compulsive animals and testing the abilities of potential new pharmacotherapies to reduce drug choice percentage within this compulsive subset may prove fruitful for finding addiction treatments and discovering neurological correlates underlying alcoholism.

Disclosures: R.S. Dulman: None. **E. Augier:** None. **H. Sun:** None. **M. Heilig:** None.

Poster

428. Alcohol: Intake and Preference

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA019682

NIH Grant AA020914

Title: Relapse to alcohol seeking in rats: The role of alcohol priming and varenicline pretreatment

Authors: *P. A. RANDALL[1], A. A. JARAMILLO[1], M. MASCIELLO[1], J. BESHEER[1,2]
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Abstract: Alcohol use disorders (AUDs) pose one of the greatest health risks worldwide. Furthermore, less than 25% of people with AUDs actually seek treatment. Those who do seek treatment or attempt to stop on their own face high relapse rates. For this reason, a great deal of work has focused on the factors that lead to relapse. One area of interest is examining the effects of alcohol priming on self-administration behavior. Previous work in rats has shown that a low priming dose of alcohol is capable of increasing responding in reinstatement trials following extinction. Additionally, there is a great deal of interest in pharmacological interventions for those struggling with alcohol abuse. Currently, the approved pharmacotherapies for alcoholism have varying success rates. Recently, there have been studies suggesting that varenicline (Chantix), which is commonly prescribed for smoking cessation, could be useful in helping patients to decrease alcohol consumption as well. Studies in rats trained to self-administer alcohol showed that varenicline decreased responding for alcohol and blocked cue-induced reinstatement. The current experiments were designed to assess both the effects of alcohol priming and whether or not varenicline was capable of blocking these effects under several conditions. The first experiment assessed the ability of an alcohol priming dose (0.5,1.0 g/kg, IG) to potentiate operant alcohol self-administration under normal conditions (non-extinction). Next, the ability of alcohol pretreatment (i.e., priming) to potentiate responding under "probe-extinction" (non-reinforced sessions) was assessed. Finally, the effects of alcohol priming in rats that had undergone extinction were examined. Furthermore, in each experiment, the effects of varenicline (1.0,3.0 mg/kg, IP) pretreatment with or without priming were assessed. Pretreatment with alcohol alone did not significantly increase alcohol-lever responses in any group. However, pretreatment with varenicline (1.0 mg/kg) alone significantly increased alcohol-lever responses under both normal and "probe-extinction" conditions. Conversely and similar to previous studies, the high dose of varenicline (3 mg/kg) decreased alcohol-lever responses regardless of whether rats had received an alcohol priming dose or not. Taken together, these findings suggest that under certain conditions priming will not necessarily lead to potentiation or reinstatement of alcohol seeking behavior. Furthermore, varenicline at low doses may actually increase alcohol

seeking behavior as opposed to reducing it, suggesting a possible priming effect on its own.
Grants: AA019682; AA020914

Disclosures: **P.A. Randall:** None. **A.A. Jaramillo:** None. **M. Masciello:** None. **J. Besheer:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Ministerio de Ciencia e Innovación of Spain (SAF2009-08136)

Title: Pleiotrophin differentially regulates the rewarding and sedative effects of ethanol

Authors: ***G. HERRADON**[1], C. PEREZ-GARCÍA[1], M. FERRER-ALCÓN[2], M. URIBARRI[2], M. VICENTE-RODRÍGUEZ[1]
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Abstract: Pleiotrophin (PTN) is a neurotrophic factor with important roles in dopaminergic neurons. PTN is upregulated in different brain areas after administration of different drugs of abuse such as amphetamine and delta-9-Tetrahydrocannabinol. In addition, we now demonstrate that acute ethanol (2 mg/kg, i.p.) administration causes a significant upregulation of PTN mRNA levels in the mouse prefrontal cortex, suggesting the possibility that endogenous PTN could modulate behavioural responses to ethanol. To test this hypothesis, we have now studied the behavioural effects of ethanol in PTN knockout (PTN-/-) mice, in mice with cortex- and hippocampus-specific transgenic PTN overexpression (PTN-Tg) and in wild type (WT) mice. We have tested the rewarding effects of ethanol in a biased conditioned place preference (CPP) apparatus and subject assignment using a 3-day conditioning protocol. Ethanol (1 and 2 g/kg) induced a significantly enhanced CPP in PTN-/- mice compared to WT mice, suggesting that PTN prevents alcohol rewarding effects. Accordingly, the conditioning effects of 2 g/kg ethanol were completely blocked in PTN-Tg mice. In addition, we tested the sedative effects of ethanol (3.6 mg/kg) in a loss of righting reflex (LORR) paradigm and the effects of ethanol on motor coordination in the rotarod test. We did not find differences between genotypes in the rotarod test suggesting endogenous expression of PTN do not modulate the ataxic effects of ethanol. On the

other hand, WT and PTN-/- mice showed a similar amount of time to recover the righting reflex whereas LORR was strikingly absent in PTN-Tg, suggesting upregulation of PTN levels in cortex and hippocampus significantly prevents the sedative effects of alcohol. Our data demonstrate that PTN differentially regulates ethanol-induced sedative and rewarding effects and suggest potentiation of the PTN signaling pathway as a promising therapeutic strategy in the treatment of alcoholism.

Disclosures: G. Herradon: None. **C. Perez-García:** None. **M. Ferrer-Alcón:** None. **M. Uribarri:** None. **M. Vicente-Rodríguez:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: Ministry of Food and Drug Safety Grant 14182MFDS979

KIT Grant KK1401-02

Title: Effects of MPEP on the expression and reinstatement of ethanol conditioned place preference in mice

Authors: *S. YOON[1], J.-Y. LEE[1], J. OH[2], E. CHOE[2], J.-W. SEO[1]
[1]Safety Pharmacol. Ctr., Korea Inst. of Toxicology, Daejeon, Korea, Republic of; [2]Dept. of Biol. Sci., Pusan Natl. Univ., Busan, Korea, Republic of

Abstract: It has been known that the glutamatergic system plays a vital role in regulating neurobehavioral effect of various abused drugs. In the present study, we evaluated the effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective antagonist of the type 5 metabotropic glutamate receptor (mGluR5) on the rewarding effects of ethanol in the conditioned place preference (CPP) paradigm. Mice were conditioned with saline or ethanol (20% v/v, 2 g/kg) for 8 consecutive days and MPEP was administrated 10 min before start of testing. In the separate experiment, ethanol-induced reinstatement was examined based on the ethanol-induced conditioned place preference. After the extinction phase, mice were pretreated with MPEP 10 min prior to a priming injection of 2.0 mg/kg ethanol. Results showed that the mGluR5 antagonist MPEP was found to significantly reduce the expression and reinstatement of ethanol-

induced conditioned place preference. These finding provide further support for a role of the mGluR5 receptor in the rewarding properties of ethanol.

Disclosures: **S. Yoon:** None. **J. Seo:** None. **J. Lee:** None. **J. Oh:** None. **E. Choe:** None.

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: AA020930

AA020929

AA018776

AA016667

Title: Influence of KCNN and other genes related to intracellular calcium signaling on drug addiction, risk for alcohol dependence, and excessive alcohol consumption

Authors: A. E. PADULA[1], M. F. LOPEZ[2], N. S. MCGUIER[1], E. J. CHESLER[3], M. F. MILES[4], *P. J. MULHOLLAND[1]
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Abstract: Small-conductance Ca2+-activated K+ (KCa2) channels regulate neuronal excitability and synaptic plasticity and have recently been implicated in alcohol and drug addiction. However, it is unknown if there is a genetic influence of the genes that encode KCa2 channels (KCNN1-3) on addiction. To evaluate the plausibility of a role for the family of KCNN genes in the behavioral effects of alcohol and other drugs of abuse, a GeneWeaver database search was performed to identify previous whole genome studies in which KCNN1-3 has been associated with alcohol- and drug-related behaviors. The family of KCNN genes was found in multiple human and rodent gene sets related to alcohol, nicotine and illicit drug addiction. This search showed that exposure to alcohol, opiates, or nicotine also significantly altered expression of KCNN1-3 in the nucleus accumbens (NAc), superior frontal gyrus, or basolateral amygdala. Because KCNN3 was found in gene sets for alcohol, cocaine, heroin, methamphetamine, and

nicotine, we further analyzed other genes in these QTLs and gene sets to identify if they were highly-related to alcohol and drug addiction. Only eight other genes were identified in 70% or more of these gene sets. To identify if specific signaling pathways were present, these genes were further analyzed with an over-representation test using PANTHER software. Intracellular Ca2+-sensing and calmodulin binding genes were significantly over-represented in this gene network. We wanted to further explore the relationship between KCNN genes and alcoholism because KCNN3 was found to be located in a gene set for an alcoholism susceptibility locus on human chromosome 1. The genetically diverse BXD recombinant inbred strains of mice consume varying amounts of alcohol, and linear regression analysis demonstrated that Kcnn3, but not Kcnn1 or Kcnn2 RMA values in the NAc significantly predicted baseline drinking levels in this panel of mice. Kcnn3 RMA levels in the NAc predicted voluntary alcohol drinking in non-dependent BXD strains, and the strength of the predictability of Kcnn3 was enhanced in alcohol-dependent mice. Elevated transcript expression levels of Kcnn3 in BXD strains were protective against dependence-induced escalation of drinking. Findings from this integrative genomics approach provide strong evidence implicating the family of KCNN genes in alcohol, nicotine and illicit drug addiction and dependence. Moreover, genes related to intracellular Ca2+ signaling and KCa2 channel function are positional candidates that may contribute to substance abuse.

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Title: Choice as a screen for compulsive alcohol drinking in rats

Authors: *E. AUGIER, R. S. DULMAN, M. HEILIG
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Abstract: Alcoholism can be defined as compulsive alcohol use that is excessive and difficult to control despite negative consequences. A critical problem in current addiction research is to understand the transition between controlled and compulsive alcohol use. While only a minority of human drug users make the transition to addiction, nearly all rats successfully acquire self-

administration, a traditional rodent model of drug abuse. Recently, we developed a mutually exclusive discrete choice procedure where rats were proposed to choose between cocaine and a non-drug alternative (i.e. water sweetened with saccharin) and found that most of them prefer the alternative reward over cocaine. Here, we thought to generalize this procedure to alcohol. We first trained non-food-deprived rats to lever press for 20% ethanol (EtOH) on a fixed-ratio-2 reinforcement schedule for several weeks. After stabilization, rats were proposed to choose between 20% EtOH and saccharin (0.04% and 0.2%), a non-caloric sweetener. We observed that, when facing a choice between alcohol and saccharin, most alcohol self-administering rats abstain from alcohol in favor of the non-drug pursuit. Moreover, the preference for the alternative reward is dose-sensitive. Interestingly, only a minority (around 15 %) of animals, a subset that aligns with human addiction rates, continues to take the drug despite the opportunity of making a different choice. Finally, naltrexone, a FDA-approved medication for alcohol dependence successfully decreased alcohol choice in a subpopulation of drug preferring rats. The results indicate that this minority of alcohol-preferring rats could represent a better animal model of alcoholism and that the choice model could be useful to better screen for compulsive animals, testing the abilities of potential new pharmacotherapies to reduce alcohol drinking and may help us elucidate the neural substrates that underlie alcoholism.

Disclosures: **E. Augier:** None. **R.S. Dulman:** None. **M. Heilig:** None.

Poster

428. Alcohol: Intake and Preference

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Title: Social isolation during adolescence increases voluntary ethanol intake via an altered circadian drinking phenotype

Authors: *J. B. PANKSEPP, E. D. RODRIGUEZ, A. E. RYABININ
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Abstract: In humans concurrent exposure to ethanol (E) and sweeteners such as saccharine (S) is commonplace during adolescence. Here we modeled this E+S connection in adolescent mice to examine whether a voluntary drinking phenotype is sensitive to differential housing (same-sex pair vs. isolate) in 4 genetically diverse strains (BALB/cJ, C57BL/6J, FVB/NJ & MSM/ms) that exhibit differences in laboratory tests of social affiliation. Using a 2-bottle choice, ascending concentration procedure (4 d each of H2O, 3E+0.2S, 6E+0.2S, 10E+0.2S & 10E-only), we compared males and females of these 4 strains to age-matched groups that received S-only exposure (i.e., 4 d each of H2O 0.2S, 0.2S, 0.2S & 10E-only). Isolate-housed (Ih) mice from all of the strains and both sexes consumed more E+S than age-matched socially housed (Sh) mice, and this effect of Ih vs. Sh persisted in a majority of the groups when the bottles were switched to 10E-only. The influence of Ih vs. Sh was not specific for S per se, as mice in the E+S only groups regulated their voluntary drinking differently than those in the S-only groups. Ih mice (from the 'S-only' groups) in all strain-by-sex combinations also increased E intake when their bottles were switched to 10E-only. Following the 10E-only phase, bottles in all groups were switched back to 10E+0.2S and blood E concentrations (BEC) were measured 4 h into the dark phase. Across the 256 mice that were sampled BECs were 90.6±67.4 mg% (mean±s.d.), indicating substantial intoxication in many individuals. However, compared to 10E+S intake, Ih induced increases in BEC were less consistent across the comparison groups. Subsequent lickometer studies confirmed that Ih in adolescent (FVB/NJ) mice resulted in increased 10E+0.2S intake. Circadian analysis revealed that Sh and Ih mice licked at comparable levels during the transition to 'lights off', with high lick rates persisting 4h into the dark phase. However, Ih mice exhibited higher levels of licking further into the dark phase, exhibiting a pronounced bout of 10E+0.2S drinking ~8.5h into the dark phase. BECs of Ih mice were significantly higher than Sh mice at this circadian time point. Collectively our results demonstrate that voluntary E+S drinking by adolescent mice is substantially greater during Ih, and that this social influence is relatively insensitive to genetic differences. Our findings also indicate that this effect of social housing is attributable to Ih mice drinking at circadian times when Sh mice do not, which may be useful in modeling the aberrant temporal drinking patterns that characterize some alcohol abuse disorders.

Disclosures: J.B. Panksepp: None. **E.D. Rodriguez:** None. **A.E. Ryabinin:** None.

Poster

428. Alcohol: Intake and Preference

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Title: Conditional knockout of Mecp2 in midbrain dopamine neurons results in increased anxiety-like behavior and promotes voluntary ethanol drinking

Authors: *M. QIANG, Z. LIU, J. LIU, X.-Y. LU, W. ZHANG
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Abstract: Methyl CpG binding protein 2 (MeCP2) is essential for the normal function of nerve cells. Since the consequences of the MeCP2 loss of function mutation in the brain are complex and involved in various effects in different brain regions and multiple neuronal systems, this protein may plays an important role of epigenetic regulation in a cell type-specific manner. Recent study in our laboratory showed that MeCP2 mediates chronic intermittent ethanol induced long-lasting DNA demethylation in the NR2B promoter. To understand the role of MeCP2 in dopamine neurons in regulating the development of alcohol drinking disorders, we generated a line of conditional knockout mice lacking MeCP2 selectively in dopamine neurons. $Mecp2^{lox/lox}$ mice were crossed with a dopamine transporter (DAT, Slc6a3) promoter-driven Cre transgenic (DAT-Cre) mouse line. Twenty male $fMeCP2^{DAT\text{-}Cre}$ offspring, i.e., lack of MeCP2 in dopamine neurons, and thirty littermate control mice were used. Mutant mice exhibited normal body weight and food consumption. Alcohol dependence was assessed by measuring voluntary ethanol drinking using a two-bottle choice test with 2-h limited access to ethanol. Open field and light/dark tests were performed to assess anxiety levels in mice. We found that mice lacking MeCP2 in dopamine neurons ($fMeCP2^{DAT\text{-}Cre}$) displayed increased anxiety and ethanol consumption compared with littermate controls. Furthermore, mice were exposed to chronic intermittent ethanol (CIE) vapor, which increased ethanol intake and anxiety-like behavior in control mice but not in $fMeCP2^{DAT\text{-}Cre}$ mice. Taking together, these data suggest a role of MeCP2 in dopamine neurons in the development of alcohol drinking behavior.

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R37 AA11852

Title: Age of first exposure to alcohol influences success rate of operant ethanol self-administration induction protocols in the absence of sweetener

Authors: *S. L. ZANDY, A. VENA, J. P. VALENTA, J. M. DOHERTY, R. A. GONZALES
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Abstract: Traditional animal models of ethanol self-administration use sweetening agents during the initial training period in order to induce significant ethanol consumption by increasing solution palatability. However, recently many ethanol self-administration models have begun to omit sweeteners to avoid the potential reinforcing effects. The objective of this study was to determine the success rates of three unsweetened ethanol protocols at inducing significant operant ethanol self-administration relevant for behavioral experiments. Adolescent (n=8; body mass at first ethanol session 214±1.73 g) and adult (n=8; body mass at first ethanol session 321±1.83 g) male Long-Evans rats were given intermittent access to ethanol (20% v/v) first in overnight sessions using an operant paradigm shown to establish high levels of voluntary drinking in outbred rats. A separate group of adult rats (n=8; body mass at first ethanol session 352±3.74 g) were given access to ethanol (10% v/v) in daily sessions using a two bottle choice (2BC) paradigm. All groups were transitioned to limited access operant sessions according to each protocol once the initial induction phase was completed. Criteria for successful induction were intake of 0.30 g/kg or greater in at least 2 of 3 consecutive limited access sessions. Intermittent operant 20% ethanol successfully induced significant ethanol consumption during limited access operant sessions in at least 50% of rats (adolescent initiation: 6/8; adult initiation: 4/8). Average ethanol consumption in limited access sessions (adolescent initiation: 0.84 ± 0.11 g/kg in 30 min; adult initiation: 0.63 ± 0.09 g/kg in 30 min) was equal or greater than previous results using a sucrose-fading protocol. Interestingly, this induction protocol was more successful in rats that began drinking in adolescence. There were no significant differences in distribution of licking behavior between groups at the start of limited access operant sessions [$F(11, 88)=1.11$, ns]. However, while total consumption in limited operant sessions was similar between age groups, rats that began drinking in adolescence showed significantly increased ethanol consumption during the first five minutes compared to rats that began drinking in adulthood [$t(8)=2.92$, $p<0.05$]. In contrast, daily 10% ethanol 2BC did not induce significant ethanol consumption in limited operant sessions. Results to date suggest an intermittent operant model using 20% ethanol successfully induces ethanol consumption in more rats than using daily 2BC access to 10% ethanol, and age of first exposure to alcohol may be a critical factor for successful initiation of significant ethanol consumption.

Disclosures: **S.L. Zandy:** None. **A. Vena:** None. **J.P. Valenta:** None. **J.M. Doherty:** None. **R.A. Gonzales:** None.

Poster

428. Alcohol: Intake and Preference

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Intramural Research Program of the National Institute on Drug Abuse, NIH/DHHS, USA (GRU and FSH)

Title: Sex differences in the effects of adolescent social deprivation on alcohol consumption in μ-opioid receptor knockout mice

Authors: *Y. MORIYA[1,2], Y. KASAHARA[1,2], F. S. HALL[3], G. R. UHL[3], H. TOMITA[1,2], I. SORA[1,4]
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Abstract: Rationale: Early social experience has been consistently shown increase alcohol consumption, perhaps by influencing stress systems. However, the connection between these

effects and alcohol consumption is complex and poorly understood. Curiously, in most acute stress models alcohol consumption is most often reduced, rather than increased, as it is following chronic social isolation of adolescent rodents (isolation-rearing). This may suggest that other factors influence the interaction between stress and alcohol exposure. Genetic factors also have a substantial influence on alcohol consumption, but only a limited set of such genetic influences on stress-induced alcohol consumption have been examined. Objectives: In experimental animals, isolation-rearing has been used as an environmental intervention that alters neurodevelopment and influences alcohol drinking behaviors. This experiment was based on the hypothesis that the effects of chronic social isolation on ethanol consumption would be influenced by both sex and the functioning of μ-opioid receptor (MOP) systems. The present study assessed the effects of isolation-rearing on later ethanol intake using a two-bottle home-cage consumption (ethanol 8% vs. water) paradigm in wild-type and MOP gene knockout (KO) male and female mice. Results: Ethanol consumption was affected by sex, genotype and isolation-rearing, MOP and there interesting interactions between these factors. In socially rearing mice MOP KO reduced ethanol consumption in male mice but increased consumption in female mice. Isolation rearing had no effect in WT mice, but isolation-rearing male MOP KO mice consumed more ethanol than socially rearing male MOP KO mice, while socially rearing female MOP KO mice had greater ethanol consumption compared with isolation-reared female MOP KO mice. Conclusion: These results indicate that MOP influences ethanol consumption, but does so in quite different ways depending on sex and previous social experience during adolescent development.

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Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA019458 (Y.S.)

Title: Amoxicillin and augmentin reduce ethanol intake and increase GLT1 expression as well as AKT phosphorylation in mesocorticolimbic regions

Authors: *Y. SARI[1], S. GOODWANI[1], P. RAO[1], R. L. BELL[2]
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Abstract: Pharmacological upregulation of glutamate transporter 1 (GLT1), a principal mediator of glutamate reuptake, in mesocorticolimbic regions of the brain is associated with reduced drug and ethanol self-administration. It has been shown that administration of the beta-lactam antibiotic ceftriaxone reduces ethanol and cocaine abuse-like behaviors as well as attenuating chronic ethanol-associated reductions in central GLT1 levels. However, it is not known if these effects are compound-specific. Therefore, the present study examined the effects of two other beta-lactam antibiotics, amoxicillin (AMOX) and augmentin (AUG), on ethanol drinking, as well as GLT1 and phosphorylated-AKT (pAKT) expression levels in the nucleus accumbens (Acb) and prefrontal cortex (PFC) of alcohol-preferring P rats. After experiencing five weeks of 24h free-choice access; the P rats were given five consecutive daily i.p. injections of saline vehicle, 100 mg/kg AMX or 100 mg/kg AUG. Both compounds significantly decreased ethanol intake and significantly increased GLT1 expression in the Acb. AUG also increased GLT1 expression in the PFC. Results for changes in pAKT levels matched those for GLT1, indicating that beta-lactam antibiotic-induced reductions in ethanol intake are negatively associated with increases in GLT1 and pAKT levels within two critical brains regions mediating drug reward and reinforcement. These findings add to a growing literature that pharmacological increases in GLT1 expression are associated with decreases in ethanol, as well as drug, intake and suggest that one mechanism mediating this effect may be increased phosphorylation of AKT. Thus, GLT1 and AKT phosphorylation may serve as molecular targets for the treatment of alcohol and drug abuse.

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Poster

428. Alcohol: Intake and Preference

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Support: T32AA007468

AA016981

AA016647

AA010760

Title: The effects of Urocortin1 knockout on voluntary drinking following chronic intermittent ethanol vapor exposure in male and female mice

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Abstract: Alcohol addiction is a disorder that takes its toll on the individual and society at large. Current research endeavors are aimed at understanding the role of genetic and environmental factors in alcohol dependence. Studies have shown that systems regulating stress and appetite also contribute to alcohol consumption. Specifically, manipulations of the corticotropin releasing factor (CRF) system have been shown to regulate alcohol consumption. The mammalian CRF system includes endogenous ligands CRF, Urocortin (Ucn)1, Ucn2 and Ucn3. While most studies have focused on the role of CRF, evidence accumulates that Ucn1 also contributes to regulation of alcohol consumption. However, previous studies have only tested the role of Ucn1 in animals that are not dependent on alcohol. In the current experiment, we used Ucn1 KOs to examine the contribution of this system to dependence-induced alcohol consumption. Adult male and female KO mice and their wild type (WT) littermates were split into eight groups [female KO-Air, female KO-Ethanol, female WT-Air, female WT-Ethanol, male KO-Air, male KO-Ethanol, male WT-Air, and male WT-Ethanol]. Mice were given access to food, water, and 15% v/v ethanol via two-bottle choice for 24h for two weeks. Dependent variables included ethanol intake, ethanol preference, water intake, and food intake. During baseline measures, genotype or sex showed no differences in any of these measures. Separate groups of mice were then exposed to three days of 16h ethanol vapor + 8h air (intermittent ethanol) or air (control) exposure and then returned to home cages to monitor ethanol intake for 5-days. Following three cycles of intermittent ethanol or air exposure, followed by subsequent ethanol consumption, a few noteworthy differences were found. In general, female mice drank more ethanol/water and ate more food than males; however, males had a higher preference for ethanol. Vapor exposure had no effect on males, but females exposed to ethanol vapors showed an increase in ethanol intake. Finally, genotype only affected preference in females, with the WT mice showing increased preference compared to KOs. In males, WT mice showed increased ethanol intake/preference and food intake compared to KOs with no difference in water intake. While the effects appear complex, all comparisons that reached significance showed lower intakes in Ucn1 KOs versus WT mice, especially in males. Our results are in general agreement with claims suggesting that CRF antagonists show potential for alcoholism treatment. However, their effects are likely to be mediated by blocking the effects of no only CRF, but Ucn1 as well.

Disclosures: J.L. Gomez: None. **D.A. Finn:** None. **C. Snelling:** None. **J. Li:** None. **A.E. Ryabinin:** None.

Poster

428. Alcohol: Intake and Preference

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA13199

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NIH EB2092

Title: Independent regulation of early drinking pattern, fluid volume and ethanol dose at a bout level in male alcohol preferring (P) rat: A concentration manipulation study

Authors: *A. V. AZAROV, D. J. WOODWARD
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Abstract: Alcohol preferring P rats have been bred for high intake of and preference for 10% v/v ethanol (10E). What yet awaits clarification is whether and how new drinking patterns develop if ethanol preference changes to prevent excessive BACs with access to more concentrated solutions. Single time water (W) deprived ethanol naïve P rats were trained to drink water from pre-filled, lick-monitoring spouts for 4-5 days. Then access was provided (i) to 10E only: in 15E group for 2 days, in 20E group for 1 day, (ii) in 20E group, to W and 15% v/v ethanol (15E), for 2 days, (iii) - final choice period - access to W and final concentration of ethanol solution: in 10E group for 18 days, in 15E and 20% v/v ethanol (20E) groups for 16 days. Fluids in 0.1 cc drops were available from identical spouts in 15-hr daily sessions spanning the dark phase of normal lighting cycle with food ad lib for 8 hr in between. Lick movements and fluid deliveries were recorded with 1-ms accuracy. In final choice, daily intake reached the highest level of 5.6 g/kg in 15E group vs. 4.7 g/kg in 10E and 20E groups, ethanol preference was 75%, 83% and 55% respectively. With 10E, nearly all W was consumed at the start, with nearly 100% preference for ethanol for most of the session. With 20E, W was consumed steadily throughout a session, presumably to supplement lack of water from 20E which intake appeared limited to prevent high BACs. This is a significant learned pattern of in-choice drinking specific to ethanol concentration. In each group, nearly 100% ethanol was consumed within bouts. Time to consume 0.1 ml of ethanol was equally short with 10E and 15E but longer with 20E. With 15E, number of bouts tended to increase and inter-bout intervals (ibi-s) remained the same. With 10E and 20E, number of bouts remained the same and ibi-s tended to elongate. Independently regulated ceilings were discovered for (1) fluid volume per bout - at 6 ml/kg, on days 11-12 of final choice,

in 10E group and (2) ethanol dose per bout - at 0.6 g/kg, on days 9-10 of final choice, in both 15E and 20E groups. 15E intake is not limited by ceilings of both a fluid volume and an ethanol dose at the bout level. The results suggest that at concentrations above 10E in P rats (a) ethanol intake can increase both per day and per drinking bout, (b) intake patterns and bout parameters are modified, (c) fluid volume and ethanol dose are regulated independently at a bout level. It appears that (1) intake of ethanol in 10E is below the sought after level due to fluid volume overload and (2) concentration of 15-17% are most expedient to generate the highest levels of both daily and bout intake to further analyze neurophysiologic mechanisms of ethanol drinking and therapeutic interventions in real time.

Disclosures: A.V. Azarov: None. **D.J. Woodward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPlex, Inc..

Poster

428. Alcohol: Intake and Preference

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Topic: C.17. Drugs of Abuse and Addiction

Support: T32MH087004

Title: Intrinsic adaptations of mesolimbic dopamine neurons that mediate individual alcohol drinking behaviors

Authors: *B. JUAREZ[1], A. K. FRIEDMAN[1], S. M. KU[1], D. CHAUDHURY[1], H. ZHANG[1], E. ROSE[2], M. CRUMILLER[3], M.-H. HAN[1]
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Abstract: Alcohol use disorders create a major global health burden. A phenomenon of alcohol use is the variability of drinking behaviors among the human population. While some people are able to consume alcohol in a controlled manner, other individuals develop severe, compulsive alcohol drinking behaviors that often result in the diagnosis of an alcohol use disorder. In order to understand the neural basis of these individual alcohol drinking behaviors, we use the genetically identical, inbred C57BL/6J male mice to model low and high alcohol drinking behaviors without the confound of different gene backgrounds. After chronic access to a 24 hr,

2-bottle choice alcohol drinking paradigm, 80% of C57BL/6J male mice develop high alcohol drinking behaviors while 10% of the mice display low alcohol drinking behaviors. Using this animal model, we sought to understand how neuronal alterations of the mesolimbic dopamine reward system mediate these individual alcohol drinking behaviors. Ventral tegmental area (VTA) dopamine (DA) neurons have been implicated in alcohol reward. In order to understand the differences in neural activity of VTA DA neurons between the genetically identical low and high alcohol drinking C57BL/6J mice, we performed anesthetized *in vivo* single-unit recordings following the alcohol drinking paradigm. Surprisingly, we found that low alcohol drinking mice displayed increased firing rate and bursting properties while high alcohol drinking mice maintained firing rate and bursting properties similar to ethanol naïve (control) mice. Optogenetically mimicking the increased VTA DA activity associated with low alcohol drinking behaviors in high alcohol drinking mice effectively reduced alcohol drinking behaviors for up to 24 hours. VTA DA neurons are known to be intrinsically altered by ethanol via a number of ion channels, including those that mediate the excitatory hyperpolarization-activated cation current (Ih) and a number of inhibitory potassium (K+) currents. To determine how these currents are altered on VTA DA neurons between the two alcohol drinking groups, we performed *in vitro* whole cell recordings. Interestingly, we found that although high alcohol drinking mice maintain firing properties similar to control mice, their Ih current was dramatically reduced compared to low alcohol drinking and control mice. Additionally, we found that the low alcohol drinking mice have a reduced inhibitory K+ peak current. Further investigations are being performed to determine whether these intrinsic changes are causal to the neuronal alteration shown to drive alcohol drinking behaviors.

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Poster

428. Alcohol: Intake and Preference

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Title: Brain regional knockdown of P2X4 receptors emphasize their role in mediating ethanol intake

Authors: N. HUYNH[1], L. R. WYATT[4], L. ASATRYAN[1], M. M. YARDLEY[2], M. W. JAKOWEC[3], *D. L. DAVIES[1]
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Abstract: P2X receptors (P2XRs) are a family of cation-permeable ligand-gated ion channels (LGICs) activated by synaptically released of extracellular ATP. Of the P2XR subtypes, P2X4 is most abundantly expressed in the CNS and is highly sensitive to ethanol inhibition. Evidence from our laboratory suggests that P2X4R may play an important role in ethanol intake and behavior. In agreement, we recently reported that male P2X4R knockout (KO) mice drank significantly more ethanol transiently compared to wildtype (WT) control. Administration of ivermectin (IVM), a drug previously shown to antagonize ethanol's effects on P2X4R, significantly reduced ethanol intake in both P2X4R KO and WT mice, however the reduction was 50% less in P2X4R KO. These findings further support the important role of P2X4R in regulating ethanol behavior. To continue investigating the regional specificity of P2X4R and their contribution to ethanol intake, we have developed a validated lentiviral gene delivery method to knockdown P2X4R in specific regions of the brain. Experimental evidence suggests that ethanol leads to an increase in dopamine mainly in the mesolimbic reward path by acting on other neurotransmitters receptor systems that modulate dopamine release. We found that lentivirus-mediated reduced expression of P2X4R in the nucleus accumbens, a primary component of the reward pathway, resulted in a transient and significant increase in ethanol intake compared to saline injected controls. This result with our P2X4R KO drinking studies suggest that the transient increase in drinking behavior is due, in part, to compensatory mechanisms by other receptor systems. For example, in KO mice we also observed expression changes in the α1 subunit of GABAA receptors in brain regions associated with regulation of ethanol behavior. This suggests that the crosstalk between P2X4R and the GABAA systems may be one potential compensatory mechanism. Current work is assessing dopamine levels in lentivirus-mediated P2X4R knockdown mice using HPLC method to determine the role P2X4R play in modulating dopamine release. Studies investigating the effects of regional P2X4R on both GABAA and dopamine neurotransmission may provide insights into the role of purinergic receptors in complex behavioral circuits that are involved in modulating ethanol drinking behaviors and may serve as additional therapeutic targets.

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Poster

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CNPq-Brazil

Title: Alcohol induced DNA brain damage in rats under low doses self administration

Authors: ***M. S. NIN**[1,2], P. A. COSTA[3], J. H. Z. POLI[3], N. D. M. SPEROTTO[4], D. J. MOURA[4], H. M. T. BARROS[3]
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Abstract: Background: Repeated intermittent ethanol access by self-administration mimics the initial use of alcohol by humans whom experience multiple periods between alcohol withdrawals and relapses, representing a pattern of alcoholics. Alcohol is a neurotoxic agent that induces degeneration, however the fundamental mechanisms and its consequences underlying alcohol-mediated brain damage remain unclear. In this sense, some authors pointed that alcohol consumption can be associated with generation of reactive oxygen species (ROS) and acetaldehyde-derived DNA lesions. The aim of this study was to verify the DNA damage and ROS formation and to evaluate minor behavioral changes in acute withdrawal periods using a model of low doses of ethanol self-administration by a forced diet in rats. Methods: Male Wistar rats were subjected to an oral ethanol self-administration procedure for a forced diet, in which they were offered 6-8% (v/v) ethanol solution for a period of 21 days followed by 5 repeated 24-hours cycles between alcohol withdrawal and reinstatement of the treatment. Control animals received a control diet without ethanol adding sucrose for same periods. Behavioral changes were analyzed on consecutives ethanol withdrawal days in the open field, elevated plus-maze and forced swim tests after 6-9 hours of alcohol deprivation. After the rats were euthanized, the pre-frontal cortex, hypothalamus, striatum, cerebellum and hippocampus were dissected to perform the alkaline and neutral comet assay and dichlorofluorescein ROS test. The data are described as mean±SEM. Results: The repeated intermittent ethanol access enhanced solution intake and alcohol-seeking (36.2±1.3g; $P<0.001$). Decreased exploratory activity was observed in the open field test (67.0±3.7s; $P<0.001$) and the animals stretched attempt less in the elevated plus-maze test (4.0±0.4s; $P=0.045$). However, in the forced swim test the withdrawn rats spent less time doing immobility behavior (165.9±7.7s; $P=0.002$). The DNA damage index of brain cells was significantly higher in almost all structures evaluated in the ethanol rats than controls, such as pre-frontal cortex (314.3±26.6; $P<0.01$), cerebellum (329.0±21.6; $P=0.0007$) and

hypothalamus (324.0±28.8; P=0.0169) except in striatum and hippocampus regions which there weren't differences. The increase in ROS formation was also observed in all brain areas. Conclusions: The short repeated alcohol withdrawal significantly induced minor behavioral changes in rats, which may be associated with anxiety and depression-like state changes. On the other hand this ethanol exposure model induces an increase in DNA damage and ROS formation.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA13641

Title: Single trial conditioned place preference to ethanol depends on β-endorphin

Authors: ***E. C. BERTRAM**, B. E. DECKER, J. E. GRISEL
Bucknell Univ., Lewisburg, PA

Abstract: Though the factors leading to alcoholism are complex, there is evidence that heritable levels of b-endorphin influence the liability toward excessive drinking. For example, individuals with low levels of b-endorphin appear to be especially prone to alcohol's rewarding effects (Aguirre, et al., 1995; Froehlich, et al., 1990; Gianoulakis 2004), and opioid antagonists are used in the clinic to reduce drinking. Since ethanol increases synthesis and release of b-endoprhin, data such as these have led to an "opioid-deficiency" hypothesis, suggesting that the rewarding effects of ethanol are mediated by opioid release. We explored the relationship between b-endorphin level and reward in transgenic mice with varying levels of this peptide, employing classically conditioned place preference to an ethanol-paired context. We hypothesized that mice with higher levels of b-endorphin would exhibit higher place preference, and that knockout mice, unable to synthesize the peptide, would lack conditioned place preference to alcohol. We found that mice with b-endorphin showed significantly higher place preference to an ethanol-paired context than mice entirely lacking the endogenous opioid. Our results support the contention that b-endorphin contributes to rewarding effects of ethanol, and especially promote the idea that this peptide influences subjective rewarding properties of the drug.

Disclosures: **E.C. Bertram:** None. **B.E. Decker:** None. **J.E. Grisel:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 13641

Title: Modeling an endophenotype for susceptibility to alcoholism: Single exposure conditioned place preference to ethanol in mice

Authors: *J. E. GRISEL[1,2], J. B. BEASLEY[2], E. C. BERTRAM[1], B. E. DECKER[1], L. P. ERCOLANO[1], M. T. ETUMA[1], A. H. HAND[2], M. WHITMIRE[2]
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Abstract: Most adults consume alcohol with relative impunity, but about 10-20% of users persist (or progress) in their consumption despite mounting and serious repercussions (World Health Organization, 2011). Because individuals are differentially susceptible to developing an addictive disorder, and at least some of the variability in risk is present before the first exposure to a drug, modeling innate sensitivity is crucial to understanding the causes of addiction and developing appropriate interventions (Ray et al., 2010). Here we probe predisposing influences to the initial rewarding effects of alcohol in a novel application of the conditioned place preference paradigm. In contrast to previous studies that have all employed repeated drug administrations, we use a single moderate-dose exposure to the drug. After demonstrating robust preference for this alcohol-paired context in three strains (C57BL/6J, DBA/2J and Swiss Websters) and both sexes of mice, we show that the positive subjective state is mediated by endogenous opioids, since pretreatment with the opioid antagonist naltrexone results in aversion, rather than preference, for the alcohol-paired cues. This model validates an assay of initial sensitivity to the subjective rewarding effects of alcohol, providing a tool for basic researchers trying to elucidate antecedent factors in this complex and destructive disease.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Title: A single exposure to acetaldehyde produces robust conditioned place preference in DBA mice

Authors: *J. P. MCCAFFERTY[1], E. C. BERTRAM[2], B. E. DECKER[2], J. E. GRISEL[2]
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Abstract: Previous evidence has demonstrated that acetaldehyde, the first metabolite of ethanol, may be one of the factors in ethanol reinforcement. Both intragastric (i.g.) and intraperitoneal (i.p.) administration of acetaldehyde has been shown to induce a conditioned place preference in both rats and mice (Peana et al., 2008; Font et al., 2005). Additionally, these studies were able to block preference using acetaldehyde-sequestering agents, demonstrating that this metabolite is necessary for conditioned place preference. We were interested in comparing the rewarding effects of ethanol and acetaldehyde in a single-exposure conditioned place preference paradigm. In the present study, naïve adult male and female DBA mice were conditioned in an unbiased apparatus, utilizing two distinct floor tile patterns paired with drug or vehicle injection. The subjects were split into two groups (n=10): the first received i.p. acetaldehyde injection (40mg/kg; Sigma-Aldrich) and the other received ethanol (1.5g/kg, also i.p.). On conditioning days, immediately after the injection mice were transferred to either side compartment for 30 minutes. Half of the mice received ethanol/acetaldehyde on the first conditioning day and saline on the second, and the other half received injections in the opposite order (first vehicle, then drug). On the test day mice were tested for a preference and following saline administration by measuring the amount of time spent in each chamber during a free access 30-minute session. Both Ethanol and Acetaldehyde resulted in conditioned place preference following a single exposure, indicative of subjective rewarding effects and supporting the notion that the first metabolite of ethanol may contribute to the drug's rewarding properties.

Disclosures: **J.P. McCafferty:** None. **E.C. Bertram:** None. **B.E. Decker:** None. **J.E. Grisel:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Title: Basal Anxiety predicts subjective rewarding effects of EtOH in mice

Authors: *K. SYDNOR, B. E. DECKER, J. E. GRISEL
Bucknell Univ., Lewisburg, PA

Abstract: Anxiolytic effects of alcohol (EtOH) have been suggested to be principal factors in the initiation and maintenance of alcohol consumption. The tension-reduction hypothesis predicts that individuals who are high in anxiety would find effects of EtOH particularly reinforcing, and therefore show a higher predisposition for problem drinking (Cappell and Herman 1972; Pohorecky 1990; Gilpin, N & Koob, G. 2008)). Here we assessed the relationship between baseline anxiety and the initial rewarding effects of alcohol using a novel application of the conditioned place reference paradigm. Outbred Swiss Webster mice (adult, naïve, male and female), were screened for anxious behavior on the plus maze and light-dark box assays, divided into two groups based on an overall composite index of anxious behavior (median split). The following week mice were conditioned and tested for single-exposure conditioned place preference to 1.5 g/kg EtOH. Subjects high in anxious behavior (i.e., less time in open arms of plus maze, light area of light dark box, etc.) show a significant preference for an alcohol-paired context, while mice exhibiting low anxiolytic behavior fail to demonstrate significant EtOH-mediated reward. This data supports the hypothesis that negative reinforcing effects of EtOH may play an important role in individual differences to the subjective rewarding effects of the drug.

Disclosures: K. Sydnor: None. **B.E. Decker:** None. **J.E. Grisel:** None.

Poster

429. Alcohol: Behavioral Effects

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Title: Visual context conditioning in rats with escalated ethanol intake assessed via a three-choice conditioned place preference paradigm

Authors: *P.-K. HUANG, L. K. QUINN, A. A. CHIBA
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Abstract: The association formed between drugs of abuse and drug-related context is a consequence of repeated volitional administration of the drug in frequented settings. With the development of addiction, addicts become more attentive to drug-related stimuli and less attentive to stimuli related to natural rewards. Furthermore, drug-related stimuli can trigger cravings in humans and approach behavior in animals. The conditioned place preference (CPP) paradigm is commonly used in addiction research to assess the ability for a drug-paired context to attract an animal compared to a vehicle-paired context. However, CPP studies rarely utilize an operant conditioning protocol to model the normal process of contextual association to self-administered drugs. Rarer still are CPP studies that compare the strength of place preference between drug-paired context and natural reward-paired context in animals displaying addictive-like behavior. Characterizing the latter across various stages of contextual conditioning contributes to greater understanding of the influence of drug self-administration on associative learning. This study examines visual context conditioning induced by water, 20% sucrose solution, and 20% ethanol solution using a three-choice CPP paradigm in rats with escalated- and steady-level ethanol intake. Escalated- and steady-level ethanol intake were induced in the home cage using the intermittent-access 20% ethanol and continuous-access 10% ethanol two-bottle choice paradigms, respectively (Simms et al., 2008). The two groups of subjects were then trained, in a novel oral self-administration apparatus, to associate each of the three rewards to a distinct visual context. After conditioning, the contingencies between context and reward were manipulated. CPP of escalated and non-escalated rats was assessed during learning, extinction, reinstatement, and context reversal phases while subjects had home cage ethanol access as well as during abstinence. Thus, allowing examination of the strength of the setting in guiding behavior in a model of rodent alcoholism.

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Poster

429. Alcohol: Behavioral Effects

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Title: Impulsive choice, but not impulsive action, is stable from adolescence to adulthood and predicts ethanol drinking in male and female rats

Authors: *L. R. HAMMERSLAG[1,2], O. A. ALADESUYI AROGUNDADE[1], A. G. KAROUNTZOS[1], J. M. GULLEY[1,3,2]
[2]Neurosci. Program, [3]Dept. of Psychology, [1]Univ. of Illinois - Urbana Champaign, Champaign, IL

Abstract: It has been theorized that impulsivity is a personality trait that predisposes individuals to problem drug use, which often begins in adolescence. However, the concept of impulsivity refers to a number of independent subtypes (e.g., action and choice) and it is unclear if one subtype may be a better predictor for drug use than another. In addition, impulsivity may not be stable over time, particularly as individuals age from adolescence to adulthood. In this study we used a modified delay discounting (DD) task to assess the stability of impulsive action and choice across two testing periods. In Experiment 1, the initial test occurred in adolescence [postnatal day (P) 31-P50] and the retest occurred in adulthood (P81-P100), whereas in Experiment 2 both the test and retest occurred during adulthood (P81-P100 and P131-P150). In both cases 30 days elapsed between test and retest. During each testing period, mildly food-restricted (≥ 95% free feeding weight) male and female Sprague-Dawley rats were trained to associate one lever with a large reward (3 pellets) and the other with a small reward (1 pellet). For 2 sessions, choice of either lever resulted in a 1-s delay before pellet delivery, followed by a 20-s intertrial interval (ITI). Next, impulsive action was assessed across 3 sessions. During these sessions responses during the ITI resulted in a 5-s signaled timeout and were used to assess impulsive action. Next, impulsive choice was assessed during 5 DD sessions, where the delay to delivery of the large reward increased once per session in ascending order (5, 15, 30, 50, and 75-s). Preference for the small, immediate reward was used to assess impulsive choice. During the retest period, rats were moved to new operant chambers and the value of the levers was reversed. Twenty days after finishing the retest period, rats were allowed 15 daily drinking sessions (30-min access to 10% ethanol in 0.2% saccharin). In both experiments we found that impulsive choice was stable from test to retest, suggesting that it is unaffected by development and repeated testing. In contrast, an individual's relative level of impulsive action was stable when both test and retest occurred in adulthood but not when the first test occurred during adolescence, suggesting that impulsive action is not stable across development. Impulsive action also

decreased from test to retest in both experiments, suggesting that it is affected by repeated testing. Impulsive choice, but not impulsive action, predicted the level of ethanol consumption in both experiments. These results suggest that impulsive choice, but not impulsive action, is a stable personality trait in rats that predicts the initiation of ethanol drinking.

Disclosures: L.R. Hammerslag: None. **O.A. Aladesuyi Arogundade:** None. **A.G. Karountzos:** None. **J.M. Gulley:** None.

Poster

429. Alcohol: Behavioral Effects

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Title: Nociceptin receptor (NOP) agonists attenuate the rewarding effects of alcohol in the conditioned place preference paradigm

Authors: N. ZAVERI[1], A. HAMID[2], P. MARQUEZ[2], M. E. MEYER[1], *K. LUTFY[2]
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Abstract: Alcoholism and alcohol-related disorders are major public health and place enormous burdens on society and economy. However, a limited number of pharmacotherapeutic agents are available to treat alcohol addiction. A growing body of literature suggests that the opioid receptor-like (ORL1) receptor (also known as the nociceptin receptor or NOP) may be a potential target to treat alcohol addiction. Indeed, nociceptin/orphanin FQ (N/OFQ), the endogenous agonist of the NOP, has been shown to block the acquisition and expression of ethanol-induced conditioned place preference (CPP), which is widely used as an animal model of reward. Intracerebroventricular N/OFQ, administration has also been demonstrated to block reinstatement of ethanol CPP. Thus, in the present study, we determined the effect of a series of novel small-molecule NOP agonists on the rewarding action of ethanol using the CPP paradigm as an animal model of ethanol reward. Mice were tested for preconditioning place preference on day 1. On days 2-4, mice were treated with vehicle or one of the NOP agonists (AT-312, SCH221510), followed by saline/ethanol (2 g/kg) or ethanol/saline administration and conditioned to the CPP chambers for 15 min. Mice were then tested under a drug-free state for

postconditioning place preference on day 5. On each test day, mice were placed in the neutral chamber and allowed to freely explore the conditioning chambers through the smaller central chamber for 15 min. The amount of time that mice spent in each CPP chamber was recorded. Our results showed that agonists with high affinity at the NOP reduced or even abolished ethanol CPP compared to their vehicle-treated control groups. Together, these data illustrate that NOP agonists may be potential pharmacotherapeutic agents to treat alcoholism and alcohol-related disorders.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01AA020394-01

Title: Blockade of kappa-opioid receptors in the basolateral amygdala inhibits negative affective cue-induced excessive alcohol self-administration in rats

Authors: ***A. BERGER**, B. WALKER
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Abstract: Alcohol dependence in both humans and rodents is associated with a dysregulated kappa-opioid receptor (KOR) system. Activation of the KOR system has been implicated in the production of negative affective states and we have previously shown that alcohol consumption escalates following the presentation of a cue associated with KOR system activation. This study sought to elucidate the neuroanatomical underpinnings of cue-induced escalation of alcohol consumption and associated negative affective states. To accomplish this, male Wistar rats were trained to self-administer 10% alcohol (w/v) in an operant self-administration paradigm before being surgically implanted with bilateral intracerebroventricular (ICV) and basolateral amygdala (BLA) guide cannulae. Following recovery, animals engaged in daily SA sessions until stability of responding was achieved. Associative conditioning sessions pairing the effects of ICV infusions of the KOR agonist U50,488 and a neutral olfactory stimuli were conducted. Subsequently, the animals were allowed to continue daily alcohol self-administration sessions

until their responding stabilized, after which the KOR antagonist nor-binaltorphimine (nor-BNI), or vehicle, was infused into the BLA 48 hours prior to presentation of the cue before alcohol self-administration sessions. The results showed that relative to vehicle, nor-BNI treated rats consumed less alcohol in response to the cue. This supports the hypothesis that negative affective cue-induced escalations in alcohol consumption involve KOR functioning in the BLA. These data provide further insights into factors that promote excessive alcohol consumption and relapse for those suffering from Alcohol Use Disorders.

Disclosures: **A. Berger:** None. **B. Walker:** None.

Poster

429. Alcohol: Behavioral Effects

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R37 AA11852

NIAAA T32 AA007471

Title: Effects of naltrexone on operant self-administration of sweetened ethanol and relapse behavior in adolescent and adult male Long-Evans rats

Authors: *J. M. DOHERTY, P. CEVALLOS, C. PARK, R. A. GONZALES
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Abstract: Alcohol is among the most widely abused drug during adolescence, yet we know little about the mechanisms that drive alcohol drinking during adolescence or if the mechanisms differ from adults. The opiate receptor antagonist naltrexone reduces ethanol consumption and relapse behavior in animal models and in certain clinical populations, however, very little research exists on the effectiveness of naltrexone in adolescents. Thus we used our rat model of operant sweetened ethanol self-administration and relapse to test the ability of naltrexone to attenuate consumption of sweetened ethanol or seeking behavior for this solution. After brief training on a sucrose solution and 14 d of operant self-administration of a 10% ethanol plus 10% sucrose solution, adolescent (postnatal day 36 at 1st ethanol exposure; n=7-10/dose) and adult (n=5-7/dose) male Long-Evans rats were injected with naltrexone (0, 0.25 or 0.5 mg/kg subcutaneous)

and a progressive ratio (PR) schedule of reinforcement was used. Rats were then tested for reinstatement of lever pressing behavior under extinction conditions after 13 d of abstinence. Control rats drank only sucrose throughout the experiment (after training, 2% sucrose was used to match sweetened ethanol response rates). Results show that no age difference in ethanol intake occurred prior to the PR test (g/kg in 20 min: adolescents 1.1 ± 0.1, adults 1.0 ± 0.1). As expected, naltrexone significantly reduced ethanol intake during the PR test (g/kg; 0.60 ± 0.07, 0.36 ± 0.04, 0.36 ± 0.03 for doses of 0, 0.25 or 0.5 mg/kg naltrexone respectively). The effect of naltrexone or age on breakpoint lever press values did not reach significance, possibly due to insufficient statistical power in the current data set. During the relapse test, naltrexone dose-dependently reduced sweetened ethanol seeking behavior in both age groups. In control rats, naltrexone (0.25 g/kg) did not influence consumption of 2% sucrose during the PR test, or sucrose seeking during the relapse test. In summary, naltrexone was equally effective at reducing ethanol intake and seeking behavior in adolescent and adult rats. Based on our previous work, a lack of age effects could be due to a fraction of adolescents (approximately 1/3) that may not be responding for central effects of ethanol. Additional experiments are needed to increase the number of subjects and increase statistical power to enable firm conclusions regarding possible differences between adolescents and adults in effects of naltrexone.

Disclosures: **J.M. Doherty:** None. **R.A. Gonzales:** None. **P. Cevallos:** None. **C. Park:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: Medical Research Council Programme Grant (no. 9536855)

GlaxoSmithKline

Title: Compulsive alcohol-seeking behaviour is attenuated by inhibiting μ-opioid receptors

Authors: *C. GIULIANO[1], Y. PEÑA-OLIVER[1], R. N. CARDINAL[2], E. T. BULLMORE[2,4], C. R. GOODLETT[5], D. BELIN[3], B. J. EVERITT[1]
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R&D, Clin. Unit Cambridge, Addenbrooke's Hosp., Cambridge, United Kingdom; [5]Dept. of Psychology, Univ. of Indiana, Indianapolis, IN

Abstract: One of the key features of drug addiction is the loss of control over drug seeking and taking despite adverse or negative outcomes. A successful way to model this aspect of addictive behavior in animals has been to adapt a seeking-taking chained schedule of intravenous cocaine self-administration in which instrumental seeking responses in rats result either in the opportunity to take cocaine, or in unpredictable mild foot-shock punishment (Pelloux 2007). In the present experiments, we have further adapted this procedure using alcohol as the drug reinforcer in order to investigate compulsive alcohol seeking in re-derived alcohol preferring rats (rP rats) pre-exposed to the intermittent 2-bottle choice procedure for 12 sessions to establish their alcohol preferring phenotype. In a subgroup of animals trained subsequently to seek and take alcohol on a chained schedule of reinforcement, alcohol seeking persisted despite the unpredictable, intermittent delivery of 0.45mA foot-shock punishment. These high compulsive rats, as compared to rats in low and intermediate subgroups, designated according to the persistence of responding during the punishment contingency, also showed increased alcohol seeking behaviour under extinction-conditions (with no reward available), and increased motivation for alcohol measured under an exponential progressive ratio schedule of alcohol reinforcement. Systemic treatment with a novel selective μ-opioid receptor antagonist significantly reduced alcohol seeking behaviour specifically in high-compulsive rats, without affecting the seeking behaviour of low-compulsive rats. Abundant evidence implicates the brain opioid systems in mediating the rewarding impact of palatable food- opiates and alcohol- intake, as well as in regulating cocaine- heroin- and highly palatable food- seeking behavior in rats (Giuliano 2012, 2013): the present results extend these observations by revealing their role in mediating compulsive alcohol-seeking behaviour. This procedure we have developed provides a methodology with which to study compulsive alcohol seeking in rats. Moreover, it suggests that inhibition of opioid transmission at the μ-opioid receptor may have potential as a treatment for the prevention of compulsive alcohol seeking and consequent relapse to alcohol intake.

Disclosures: C. Giuliano: None. **Y. Peña-Oliver:** None. **R.N. Cardinal:** None. **E.T. Bullmore:** A. Employment/Salary (full or part-time):; GlaxoSmithKline R&D. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GlaxoSmithKline R&D. **C.R. Goodlett:** None. **D. Belin:** None. **B.J. Everitt:** 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.; GlaxoSmithKline R&D.

Poster

429. Alcohol: Behavioral Effects

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 429.11/CC16

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA021233

Title: Understanding circadian modulation of alcohol sensitivity in *Drosophila* melanogaster

Authors: *H. KRISHNAN, A. DENOBREGA, D. DEROSIA, L. C. LYONS
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Abstract: Alcohol abuse and alcoholism result in significant adverse health consequences to individuals and tremendous economic costs to businesses and society. Identifying and delineating the factors and mechanisms affecting behavioral and neurobiological responses to alcohol and alcohol physiology are necessary for development of therapeutic treatments and to ameliorate these costs. Given its well-characterized circadian clock and neuronal circuitry, we have used *Drosophila melanogaster* to investigate how the endogenous circadian clock affects alcohol sensitivity and how alcohol exposure affects the functioning of the circadian clock. Previously, we found that the circadian clock modulates the acute loss of motor control in flies using the loss-of-righting reflex (LoRR) assay. Flies exhibit a circadian rhythm in the LoRR with the greatest sensitivity to alcohol occurring from mid to late night (van der Linde and Lyons, 2011). However, alcohol tolerance does not appear to be regulated by the circadian clock suggesting that circadian modulation of alcohol sensitivity occurs downstream of initial absorbance. In the current research, we have extended these studies analyzing the effects of the circadian clock on additional alcohol-induced behaviors. We found that sedation also appears to be modulated by the circadian clock with flies succumbing to the sedating effects of alcohol faster when exposure occurs during the night compared to alcohol exposure during the subjective day. As sex-specific differences have been reported for circadian-alcohol interactions, we are also investigating whether the circadian clock differentially modulates alcohol-induced behaviors in male versus female flies. These behavioral studies defining the parameters of circadian modulation of alcohol-induced behaviors in *Drosophila melanogaster* provide a foundation for future mechanistic studies at the cellular and molecular levels.

Disclosures: **H. Krishnan:** None. **A. Denobrega:** None. **D. Derosia:** None. **L.C. Lyons:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020042

NIH Grant AG031687

NARSAD YI 2008

Title: Ethanol sensitivity is decreased by inhibition of phosphodiesterase-4 (PDE4)

Authors: *H. ZHANG[1], Y. XU[1], Y. HUANG[1], R. T. HANSEN[1], C. PANG[1], M. CONTI[2]
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Abstract: Ethanol sensitivity is changed in response to alteration of cyclic AMP (cAMP) signaling. Activation of PKA in the brain increases sensitivity to ethanol exposure (i.e., prolongs ethanol-induced loss of righting reflex). Mice deficient in the regulatory IIbeta (RIIbeta) subunit of PKA display less ethanol sensitivity. The regulatory role of PKA signaling in ethanol-induced sedation may differ in various species, ages or sexes. However, it is not known if phosphodiesterase-4 (PDE4), the major enzyme that catalyzes the hydrolysis of cAMP in neurons, contributes to the regulation of ethanol sensitivity. Using mice deficient in PDE4B or PDE4D and wild-type control mice, treated with or without rolipram, a prototypical inhibitor of PDE4, we determined the role of PDE4 and its subtypes in the regulation of ethanol-induced loss of righting reflex. We found that pretreatment with rolipram (0.1-1 mg/kg, i.p.) shortened the duration of sleeping induced by the hypnotic dose of ethanol in a dose-dependent manner. The effect of rolipram was age-dependent, i.e., in mice at the age of 2-8 months, the older, the longer sleeping duration or the more sensitive to ethanol exposure. Mice deficient in PDE4D, but not PDE4B, mimicked the ability of rolipram to reduce the ethanol-induced sleeping duration. No difference was observed between male and female mice. Ethanol metabolism was not altered by rolipram. Together, the results suggest that targeting PDE4, in particular PDE4D, alters sensitivity to ethanol exposure. Inhibitors of PDE4, especially its PDE4D subtype, may be beneficial for treatment of alcohol intoxication. It was noted that the finding appears not consistent with the published studies. Further studies are needed to clarify the inconsistence [This work was supported by research grants from NIAAA (AA020042) and NIA (AG031687), both to H.T.Z.].

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Poster

429. Alcohol: Behavioral Effects

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 429.13/CC18

Topic: C.17. Drugs of Abuse and Addiction

Title: The effects of alcohol consumption on prospective memory in college students

Authors: ***S. A. RASKIN**[1], S. DHALIWAL[2]
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Abstract: Prospective memory is the ability to remember to perform an action at a designated time in the future. This study examines the effects of alcohol use on prospective memory in college students by looking at the correlation between alcohol consumption and students' performance on the Memory for Intentions Test (MIST). Participants answered questions regarding the frequency of consumption, the number of drinks consumed, the effects consumption may have had, and other substances used. They were also asked to provide a daily breakdown of how much was consumed to determine if they were binge drinkers and what their maximum and minimum levels of consumption were. Following these two tasks, participants completed the MIST, where they were asked to perform certain tasks at designated times. The MIST tested for their responses using time intervals, event-based cues, time-based cues, action-based cues, and verbal-based cues. Depending on the results, methods for improving prospective memory in college students may be developed and consumption levels which cause impairment may be determined.

Disclosures: **S.A. Raskin:** None. **S. Dhaliwal:** None.

Poster

429. Alcohol: Behavioral Effects

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Program#/Poster#: 429.14/CC19

Topic: C.17. Drugs of Abuse and Addiction

Support: PIBIC/UNIANCHIETA

Title: Brain morphology, nutritional and behavioral evaluation of rats exposed to chronic alcohol intake

Authors: *T. ITIDA, SR[1], M. R. DA CUNHA[2], R. N. ISAYAMA[3], M. C. Z. CASTELLI[2]
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Abstract: Alcoholism has a tremendous impact on morphology and functioning of the nervous system. Chronic ethanol intake is a common cause for addiction in humans. Because alcoholism has a widespread multifactor interference, the present study aimed at analyzing brain morphology, nutritional and behavioral alterations resulting from chronic alcohol exposure in rats. This work occurred under the approval of the ethics` committee and all procedures were conducted to minimize suffering of animals. Adult young male wistar rats (n=16) were housed in standard cages and provided with balanced food ad libitum. A non-alcoholic control group (CRT, n=8) was compared with a group of chronic alcohol drinkers (ALC, n=8). ALC received bottles with a traditional and largely consumed alcoholic beverage in Brazil, known as cachaça. Growing concentrations of cachaça at 5, 10, 15 and 20% were administered for a week each. After this adaptation period, ALC received cachaça at 25% in drinking water for 4 months. During experimental stages, the specimens were submitted to hydric restriction to approximately 80% of their weight ad libitum, aiming the reinforcement effect of water. All animals were submitted to activities that involve prior stimuli of discriminative tasks and sensitivity to their proper behavior. Chambers equipped with a bar that releases 0.05ml drinking water when activated were used for operating conditioning tasks. An additional floor apparatus was used to create the open field environment. Both groups were monitored for food intake and body weight gain. ALC underwent a time for alcoholism induction and, four months later, all animals were submitted to behavioral tests and histomorphological analysis of their brains. Preliminary results have shown body weight mean initial (401.6 and 407.6g) and final (496.3g and 535.6g) for CRT and ALC, respectively. Histomorphological analysis revealed sings of fragmented and degenerated tissue in ALC brains, with special effect in the cortical layers. Periodic observations and recording for behavioral parameters showed hyperactivity in ALC, compared to CRT. Bearing all this in mind, it may be concluded that chronic condition of cachaça's intake can damage specific areas of the telencephalon, which was associated with body weight changes, food consumption and behavioral alterations. These findings are in accordance with preview studies on alcoholism. However, further investigations are encouraged to elucidate other specific effects regarding the use of cachaça and its relevance as a model of chronic alcoholism.

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Poster

429. Alcohol: Behavioral Effects

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Support: Funds from wayne state university department of neurosurgery (ACC)

Supported with resources and the use of facilities at the John D. Dingell VA Medical Center, Detroit, MI

Title: Decreased ethanol-induced sensitization is associated with decreased cannabinoid receptor-1 in a mouse model of postttraumatic stress disorder

Authors: *J. J. MATCHYNSKI[1,2], L. L. SUSICK[1,2], B. L. SCHNEIDER[1,2,3], S. A. PERRINE[3], A. C. CONTI[1,2]
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Abstract: The high comorbidity of alcoholism among individuals with posttraumatic stress disorder (PTSD) suggests alterations in the response of the traumatized brain to alcohol. Cannabinoid-receptor-1 (CB1) is highly localized within the striatum and functions to mediate alcohol-induced plasticity and in the hippocampus where it regulates stress. In order to investigate the involvement of CB1 in the alcohol-PTSD interaction, the present study measured the effects of traumatic stress exposure using a model of PTSD, mouse single-prolonged-stress (mSPS), on ethanol (EtOH)-induced locomotor sensitization and levels of CB1. C57Bl/6 mice were exposed to mSPS which consists of 4 consecutive stressors: a 2-h restraint, a 10-min group forced swim, a 15-min exposure to rat bedding, and diethyl ether exposure until unconscious (~5 min). After a 7-day undisturbed period, mice were assessed for behavioral sensitization to EtOH over a 10-day period. On day 1 of EtOH sensitization, mice were given saline injections (i.p.) and habituated to the locomotor activity (LMA) chambers for 1 h. The next day, 2.0 g/kg EtOH (20% v/v in saline) or saline was administered to the mice, and their LMA was recorded for 10 min. Injections of saline or EtOH (2.5 g/kg) were continued daily for 9 days for the development of sensitization. On the 10th day mice were challenged with 2.0 g/kg EtOH to evaluate the

expression of sensitization. Immediately after LMA testing on the challenge day, brains were removed and frozen. CB1 protein levels were measured in the anterior striatum using standard immunoblotting techniques. EtOH-treated control mice showed expected increases in LMA upon EtOH challenge compared to their initial EtOH exposure, and to saline-treated controls, indicating EtOH sensitization. In contrast, mSPS mice did not demonstrate behavioral sensitization to EtOH treatment, displaying significantly reduced LMA upon EtOH challenge compared to EtOH-treated control mice. The LMA response to acute EtOH was equivalent between groups. Protein analyses indicated significant decreases in CB1 in the anterior striatum in mice exposed to mSPS followed by chronic EtOH compared to saline and mSPS controls. These data indicate that mSPS exposure disrupts the typical behavioral sensitizing effects of EtOH, a response indicative of impaired neuroplasticity. Additionally, this study demonstrates that the combination of mSPS and chronic EtOH exposure leads to dramatic decreases of CB1 in the striatum, providing a mechanism of interest for understanding the effects of EtOH following traumatic stress.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA and NIMHD G12MD007592

National Institute on Minority Health and Health Disparities 8G12MD007592

Title: The role of the dopamine/ecdysteroid receptor DopEcR in behavioral disinhibition

Authors: *G. P. ARANDA[1], I. MERCADO[2], I. OLIVAS[2], P. EVANS[3], K.-A. HAN[2]
[1]UTEP, El Paso, TX; [2]Univ. of Texas at El Paso, El Paso, TX; [3]Babraham Inst., Cambridge, United Kingdom

Abstract: Alcohol exerts numerous effects on behavior through its interaction with different signaling molecules and various effector cells. Of particular interest are the molecules in the reward and cognition-related neural sites in the *Drosophila* and mammalian brains. The

physiological roles of steroid hormones and dopamine in ethanol-induced behavioral plasticity are still unclear. The novel DopEcR receptor can bind to both the neurotransmitter dopamine and the steroid hormone ecdysone to modulate intracellular signaling. This receptor represents a homolog of the vertebrate gamma adrenergic receptors. The goal of our study is to elucidate the roles and mechanism that dopamine and ecdysone exert through this membrane receptor for ethanol-induced behaviors such as hyperactivity, sedation, tolerance, sensitization and courtship disinhibition. We found that the flies deficient in or overexpressing DopEcR showed abnormal ethanol sensitivity and courtship disinhibition. We are currently investigating if the phenotypes are due to the receptor's physiological or developmental deficiency or over action. We are also conducting experiments to clarify the relative contributions of dopamine and ecdysone to the phenotypes. This work was supported by the NIAAA and NIMHD G12MD007592 grants and the National Institute on Minority Health and Health Disparities 8G12MD007592 grant.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA T32AA00745

NIAAA AA020098

NIAAA AA06420

Title: Time course of cognitive function following prolonged abstinence from chronic ethanol exposure in a model of alcohol dependence

Authors: *M. C. STAPLES, C. D. MANDYAM
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Abstract: Dependence on alcohol is a pathological condition which develops over repeated cycles of excessive alcohol consumption and periods of abstinence. Many studies have demonstrated a striking cognitive impact of excessive ethanol consumption and dependence, but to our knowledge, there has not yet been an assessment of hippocampal-sensitive cognitive

function over an extended period of abstinence following ethanol dependence. Therefore, we used a trace fear conditioning protocol to measure acquisition, retention, and persistence of emotional memories during the abstinent period. Following seven weeks of chronic intermittent ethanol vapor exposure (CIE, ethanol vapor on for 14 hours, air for 10 hours each day) and 72 hours of subsequent withdrawal from ethanol exposure, animals were trained in the cognitive task and tested 24 hours, 10 days, or 21 days later. CIE had no impact on acquisition of the task (measured as freezing during the trace periods following the tone presentation on the training day), or on the contextual recall at any of the three time points (measured as freezing during the first three minutes of the testing session). Recall of the trace memory, assessed as freezing during the first trace period on the testing day, was significantly or nearly significantly impaired by CIE at all three time points of testing (24 hours: $p = 0.04$; 10 days: $p = 0.02$; 21 days: $p = 0.08$). At 24 hours, but not at 10 or 21 days, CIE resulted in the persistence of the fear memory, with freezing during the fifth trace period not significantly different from the first trace period, while control animals at each time point demonstrated significant reductions in responding in the fifth trace period as compared to the first. Therefore, it can be concluded that as abstinence from ethanol dependence persists, some hippocampal-sensitive cognitive function returns, but some facets of emotional memory remain impaired. It is possible that cognitive function would return to levels comparable to those observed in control animals at a point beyond the scope of this initial study; future studies should be designed with abstinence periods beyond those described here to support this theory. Additionally, further investigations into the neurobiological underpinnings of this perturbed behavior should be conducted to better understand more precisely the deleterious impact of ethanol dependence.

Disclosures: **M.C. Staples:** None. **C.D. Mandyam:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA12882

Title: Model of voluntary ethanol consumption in zebrafish: Effects on behavior and orexigenic peptide gene expression in the brain

Authors: M. STERLING, O. KARATAYEV, I. MORGANSTERN, *S. F. LEIBOWITZ
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Abstract: Background: Studies in multiple species have revealed an important role for hypothalamic orexigenic peptides in stimulating the consumption of food and drugs of abuse, which in turn is found to further increase endogenous expression of these peptides. Because of the complexity of the brain and behaviors of these species, we are seeking a simpler animal model that will permit a more comprehensive analysis of the different brain peptides in multiple brain areas and their role in controlling consummatory and related behaviors. Recent studies in zebrafish have shown alcohol in the water to affect brain neurotransmitters, such as serotonin and dopamine, and a variety of behaviors possibly mediated by these neurochemicals. While building on these findings, the goal of the present investigation was, first, to develop a zebrafish model of voluntary alcohol consumption similar to that used in rat studies and, second, to determine whether alcohol consumed by the zebrafish affects the expression of orexigenic peptides orexin/hypocretin (OX) and galanin (GAL) and induces behaviors in a manner similar to that observed in the rat. Methods: Adult zebrafish (AB) (n=15/group) were trained to consume gelatin containing 10% or 20% alcohol (v/v) or gelatin containing no alcohol (control). Once trained, the effect of alcohol consumption on locomotion, novelty seeking, and aggressive behavior was examined. To measure the effect of alcohol consumption on peptide expression, the hypothalamus was dissected and analyzed for GAL and OX mRNA expression using quantitative real-time PCR. Results: We found that zebrafish voluntarily consume gelatin that contains 10% or 20% alcohol and that the amount consumed is strongly, positively correlated (r=+0.80) with their blood alcohol levels. This consumption of alcohol compared to control gelatin produces significant behavioral changes, which include increased locomotor activity, novelty-seeking and aggressive behavior. Further, these behavioral changes are accompanied by increased mRNA expression of the orexigenic peptides, OX and GAL, in the hypothalamus. Conclusions: These results demonstrate for the first time that voluntary consumption of alcohol can be studied in zebrafish and that alcohol consumed in this manner produces behavioral and hypothalamic peptide changes very similar to that described in the rat. These results suggest that zebrafish voluntarily consume enough alcohol to be pharmacologically relevant.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01AA021121

Title: The impact of Pavlovian cues and adolescent alcohol exposure on inter-temporal choice

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Abstract: Early life alcohol use is a major public health concern, in part, because it has been reliably associated with increased risk for life-long substance abuse. Indeed, it has been widely observed that individuals with a history of chronic alcohol abuse show an impaired ability to make adaptive decisions in both their daily lives and in lab settings. We have previously demonstrated that rats with a history of adolescent alcohol use make more risky choices as adults, mirrored by changes to phasic dopamine transmission in response to cues that predict risky choices, which may be attributable to a selective imbalance in reinforcement learning where the assignment of value to Pavlovian cues is enhanced by alcohol exposure. In the current work, we examined two interrelated hypotheses examining the impact of Pavlovian cues and adolescent alcohol exposure on decision making. First, we examined inter-temporal choice to assess if our previous results on uncertainty-based decision making generalize to other forms of choice behavior that may involve impulsivity. Second, given the role of phasic dopamine signaling in the encoding of Pavlovian cues and the impact of alcohol exposure on this type of learning, we tested the prediction that the introduction of such cues may increase impulsive choice in normal rats and be exacerbated by adolescent alcohol exposure. Adolescent (postnatal day 30 to 49) male Sprague-Dawley rats were exposed to 20 days of voluntary access to an ethanol (10%) or control gelatin. Following withdrawal, rats were bilaterally implanted with microelectrodes for detection of dopamine by fast-scan cyclic voltammetry in the nucleus accumbens core. Value-based decision making was assessed in adulthood (postnatal day 80 to 99) using an inter-temporal choice task, in which rats choose between a small, immediate reward and a larger, delayed reward. To assess the impact of Pavlovian cues on choice behavior, separate groups were trained to initiate trials in response to discrete cue presentation or to self-initiate trials in the absence of a discrete cue based on the passage of time. The results indicate that animals given alcohol during adolescence exhibit a decreased tolerance to increasing delay times for high rewards relative to controls, demonstrated by a steeper discounting curve across sessions. Similarly, control animals that were cued at the start of each trial showed steeper discounting curves relative to uncued animals, similar to that of alcohol-treated animals. These findings demonstrate that adolescent alcohol exposure leads to maladaptive decision-making strategies in adulthood and extend this work by examine the role of Pavlovian cues in these behaviors.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: FAPESP 2010/18066-0

Title: Ziprasidone and social interaction accelerate the onset of extinction of ethanol-induced conditioned place preference

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Abstract: Drug addiction is critically associated with the development of conditioning of the drug reinforcing effects with the environmental cues surrounding drug use. Thus, extinction of drug-environment conditioning is critical to the development of an effective treatment for drug addiction. Recently, we have demonstrated that the administration of antipsychotics or social interaction with an antipsychotic-treated conspecific can attenuate behavioral responses induced by drugs of abuse in mice. Here, we investigated the effects of an antipsychotic (ziprasidone) administration combined with social interaction with an antipsychotic-treated conspecific on the extinction of already established ethanol-induced conditioned place preference (CPP), an animal model that measures the rewarding properties of drugs. Thirty-six 3-month-old male Swiss mice were subjected to the CPP procedure with 1.8 g/kg ethanol (ETH). An unbiased design was used and conditioning was performed 2 sessions/day (6 h apart) for 4 days. Following the CPP test, animals were allocated to 3 groups (N=12), and subjected to the extinction procedure: ETH-VEH - extinction with vehicle alone, ETH-ZIP - extinction with 10 mg/kg ziprasidone alone, and ETH-ZIP+ZIP - extinction with ziprasidone and a conspecific also treated with ziprasidone. These extinction sessions consisted of 3-day cycles and were performed 3 times (Ext1, Ext2 and Ext3). Briefly, animals received VEH or ZIP, alone or with a ZIP-treated conspecific, associated with the ETH-paired compartment for 2 days. VEH was associated with the other compartment. On the 3rd day of the extinction cycle, animals were tested for their preference for each compartment of the apparatus. The results showed that: 1) ETH administration induced a robust

CPP (effect of compartment, two-way ANOVA), 2) All of the groups extinguished ETH-induced CPP (effect of days, ANOVA with repeated measures), and 3) Ziprasidone administration in the drug-paired compartment, alone or in combination with a ziprasidone-treated conspecific, accelerated the onset of extinction. Thus, while the ETH-VEH group showed no significant modifications in the time spent in the ETH-paired compartment after all the extinction cycles, the ETH-ZIP and ETH-ZIP+ZIP groups presented a reduction in the time spent in the ETH-paired compartment from Ext1 onwards (SNK post hoc test). We conclude that ziprasidone treatment, either combined or not with social interaction, in the same environmental context previously associated with ETH, can facilitate extinction of ETH rewarding effects. These experimental results provide important insights for the treatment of alcoholism in humans.

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Poster

429. Alcohol: Behavioral Effects

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Support: Fonds de recherche du Québec – Santé

NSERC 387224-2010

Concordia University

Title: Autoshaping with ethanol in rats: A shift from goal-tracking to sign-tracking

Authors: *J.-M. N. MADDUX, C. S. SREY, N. CHAUDHRI
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Abstract: Pavlovian conditioning processes can operate in the initiation and maintenance of alcohol use in humans. It is thus important to develop preclinical laboratory models of Pavlovian conditioned responding to alcohol-associated cues, in order to study the behavioral and neural processes that contribute to the role of Pavlovian learning in addiction in humans. Here we report the observation of sign-tracking behavior to an ethanol-associated cue in rats. Male Long-Evans

rats (Harlan) were first acclimated to the taste and pharmacological effects of 15% ethanol (v/v) in their home cages using an intermittent access two-bottle choice procedure, and then trained in operant conditioning chambers using a Pavlovian autoshaping paradigm in which a 10-second presentation of a retractable lever served as the conditioned stimulus (CS) and 15% ethanol served as the unconditioned stimulus (US) (0.2 ml ethanol/CS trial; 12 trials/session). Goal-tracking, as measured by entries into the fluid port where ethanol was delivered, developed rapidly. With extended training, goal-tracking responses waned, and sign-tracking responses, as measured by lever presses, emerged. A control group that received equal presentations of the lever CS and ethanol US but in an explicitly unpaired fashion did not show goal-tracking or sign-tracking responses throughout training, indicating that an associative CS-US relationship is necessary for the development of these conditioned responses. Finally, in an operant test of conditioned reinforcement, rats were allowed the opportunity to earn presentations of the lever that had previously served as the CS in the autoshaping phase of the experiment. Two new response manipulanda (nosepoke apertures) were made available for the test: nosepokes in the active aperture resulted in a brief 2.5-second presentation of the lever, whereas nosepokes in the inactive aperture had no consequences. Rats that had received CS-US pairings during autoshaping training showed discriminated nosepoke responding in the conditioned reinforcement test, with more active relative to inactive nosepokes, whereas rats in the unpaired control group did not. These findings emphasize that alcohol-associated stimuli can exert powerful behavioral control, and open up future lines of research to identify the pharmacological and neural substrates underlying these behaviors.

Disclosures: **J.N. Maddux:** None. **C.S. Srey:** None. **N. Chaudhri:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Kappa-opioid receptor antagonism in the nucleus accumbens dissociates escalated alcohol consumption and negative affect from physiological withdrawal in alcohol-dependent rats

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Abstract: Alcohol dependence is accompanied by physiological withdrawal symptoms and negative affect during withdrawal, which has been hypothesized to be the basis for an alcohol self-medication hypothesis where alcoholics escalate their alcohol use in order to alleviate withdrawal. Therefore, attenuation of withdrawal-induced negative affect could reduce escalated alcohol consumption. The kappa-opioid receptor/dynorphin (KOR/DYN) system has been implicated in alcohol withdrawal-induced escalated drinking and negative affect. Specifically, KOR-antagonists attenuate both escalated alcohol drinking and negative affect in dependent rodents. Previous data from this lab has demonstrated that the KOR antagonist nor-binaltorphimine (nor-BNI) reverses alcohol withdrawal-induced escalation of rat 22-kHz ultrasonic vocalizations (USVs) when administered into the lateral ventricles. Measurement of 22-kHz USVs is an ethologically valid strategy used to assess negative affective states in rats. Previous evidence has implicated KORs in the nucleus accumbens (Acb) shell in escalated alcohol consumption observed in alcohol dependent rodents during acute withdrawal. Therefore, this study aimed to assess the effects of intra-Acb nor-BNI on escalated alcohol self-administration, USVs and physiological withdrawal signs during acute withdrawal. Male Wistar rats were trained to self-administer alcohol, after which Acb shell guide cannula were surgically implanted. After recovery, animals underwent dependence induction via inhalation of intermittent alcohol vapor for 4 weeks. Following dependence induction, rats were again allowed to self-administer alcohol during acute withdrawal until stability was achieved. Nor-BNI (0, 2, or 6 ug) was infused into the Acb shell 5 minutes prior to alcohol self-administration. Nor-BNI dose-dependently decreased escalated alcohol consumption and 22-kHz USVs, but had no effect on physiological withdrawal scores. The results demonstrate that KOR/DYN activation in the Acb in alcohol dependent rats is necessary for escalated withdrawal-induced negative affect-like states and escalated alcohol self-administration, but not escalated physiological withdrawal signs. These data further demonstrate that the neurobiological substrates of motivation/negative affect that drive maladaptive behavioral phenotypes in alcohol dependence can be dissociated from those related to physiological withdrawal and provide possible strategies for developing novel pharmacotherapies to treat alcohol use disorders.

Disclosures: **A. Williams:** None. **B. Walker:** None.

Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Title: Circadian influences on the anxiolytic effects of alcohol in DBA mice

Authors: *J. NELSON, C. NELSON, J. E. GRISEL
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Abstract: Time of testing during the circadian cycle in lab animals varies across and even within labs. Previous studies have examined the effects of circadian rhythm on cognitive and locomotor tests in mice, with conflicting results (Roedel et al. 2006; Munn et al. 2011). Circadian cycle affects many systems within an organism, and it was the goal of this study to examine circadian influences on the anxiolytic effects of alcohol in DBA mice. In the current study, measures of anxious behavior were taken using the elevated plus maze and light-dark box during two periods of the circadian cycle, 6 hours into the active phase and 10 hours into the inactive phase. Overall, there was a significant effect of circadian cycle on anxious behavior and locomotor activity, with subjects tested during the active phase showing less anxious behavior and more locomotor activity compared to those tested during the inactive phase. Specifically, alcohol had a larger effect on anxious behavior and locomotor activity during the active phase than during the inactive phase. The results from this study highlight the significant effects of circadian rhythm behavior in mice.

Disclosures: J. Nelson: None. **C. Nelson:** None. **J.E. Grisel:** None.

Poster

429. Alcohol: Behavioral Effects

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Program#/Poster#: 429.24/CC29

Topic: C.17. Drugs of Abuse and Addiction

Support: AA019682

Title: Reduced interoceptive sensitivity to alcohol following stress hormone exposure in rats: Identification of potential novel brain regional involvement

Authors: *A. A. JARAMILLO[1], S. FRISBEE[2], P. A. RANDALL[2], J. BESHEER[2]
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Abstract: Stressful life events and chronic stressors are associated with escalations in alcohol drinking. Stress leads to the secretion of corticosterone (CORT) in the rodent. Chronic exposure to corticosterone (CORT) in the drinking water (which models a period of heightened elevations in CORT) results in reduced sensitivity to the interoceptive/subjective effects of alcohol and increased alcohol self-administration in rats. Previous work has demonstrated a role for the nucleus accumbens (Acb) core in regulating these alcohol-related behaviors. Therefore, we sought to investigate additional brain regions involved in mediating sensitivity to alcohol. Male Long Evans rats (n=13) received a microinjection of the retrograde tracer Fluorogold directed at the Acb core. The Acb core afferent projections were visualized through immunohistochemistry. Dense Fluorogold immunoreactivity (IR) was found in the insular cortex and in the reuniens/rhomboid thalamic nuclei. Next, we sought to examine whether response to alcohol was altered in these brain regions following CORT exposure.Following CORT exposure (7 days; 0.3 mg/ml CORT in the drinking water), the rats (n= 28-32) were administered alcohol (1 g/kg, IG) or water (IG). 90 minutes later, the rats were sacrificed and brain tissue was processed for c-Fos IR. An overall increase in c-Fos IR was observed in the insular cortex following CORT exposure. In addition, CORT exposure attenuated an alcohol-induced increase in c-Fos IR in the reuniens/rhomboid nuclei. Next, to begin to examine a possible role of these brain regions in modulating sensitivity to alcohol, we examined neural activation in male Long Evans rats (n=8) trained to discriminate the interoceptive effects of alcohol (2 g/kg, IG) vs. water (IG) in a standard 2-lever operant task. After acquisition of the discrimination, rats were administered alcohol (2 g/kg, IG) or water (IG) and underwent a discrimination test session. A significant alcohol-induced increase in c-Fos IR was observed in the insular cortex and the reuniens/rhomboid nuclei. Thus, neural activation in the insular cortex and the reuniens/rhomboid nuclei suggests that these brain regions may play a role in modulating sensitivity to alcohol. Further, based on our previous research, these data may implicate the recruitment of a striatal-insular/thalamic circuit in modulating the interoceptive effects of alcohol and may suggest altered activity of this circuit following episodes of chronic elevation in CORT.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA013476

Title: Acute behavioral effects of alcohol in TLR4 knock-out rats

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Abstract: Toll-like receptors (TLRs) are part of the innate immune system and are activated in chronic inflammatory diseases including those that affect the CNS. TLR4s are found in glial and macrophages in the CNS and are involved in alcohol-induced neuroinflammation. TLR4s also recognize lipopolysaccharide (LPS) that causes neuroinflammation and enhances alcohol drinking in mice. To further assess the role of TLR4 in the behavioral effects of alcohol, we tested TLR4 knock-out rats to determine if deletion of this gene affected alcohol-induced locomotor activity and motor coordination. Six heterozygous breeding pairs were used to generate knock-out (KO), hetereozygous (HET), and wildtype (WT) offspring. Tests were conducted in male and female adult rats and included assessments of locomotor activity measured for 120-min after alcohol (1.5 g/kg; PO) administration and alcohol-induced coordination in the rotorod tested at 30- and 120-min post alcohol (and water) administration. Initial analyses shows no effect of genotype on any measure of locomotor activity although alcohol enhanced locomotor activity more in female rats than male rats. Female rats also showed greater motor coordination on the rotorod after water and alcohol administration at both time points compared to male rats. Genotype interacted with the sex effect on motor coordination when tested 120-min post-alcohol administration. Female KO rats showed greater motor impairment than female WT at both 30- and 120-min post-alcohol administration whereas there was no differences in motor ability between male KO and male WT rats. Tests of anxiety using elevated plus maze have been conducted and data are being analyzed. Additionally, alcohol-induced withdrawal will be examined. Thus, preliminary data suggest that the contribution of toll-like receptor 4 to the acute effects of alcohol are sex-dependent.

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Poster

429. Alcohol: Behavioral Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01AA07112

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Title: Alcoholics' responses to changing reward contingencies in a probabilistic reversal learning fMRI task

Authors: *S. M. RUIZ[1,2], K. S. SAWYER[1,2], E. VALERA[3,4,5], S. LEHAR[1,2], M. VALMAS[1,2], P. L. REMIJNSE[6], G. J. HARRIS[3,4,5], M. OSCAR-BERMAN[1,2,3]
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Abstract: Background: Alcoholism may be linked to functional deficits in neural systems that guide motivated behaviors, including regions that are sensitive to reinforcement. The aim of the present study was to assess the neural underpinnings of alcoholic participants' propensity to modulate behavior as a function of reward. To this end, we employed a reversal learning task (Remijnse et al., 2005) sensitive to the ability to learn changing stimulus-reinforcement associations. Methods: In this study, 29 abstinent alcoholic individuals (14 women) and 22 demographically similar nonalcoholic controls (11 women) completed a probabilistic reversal learning task during functional magnetic resonance imaging (fMRI) at 3T using a 12-channel head coil. Participants were asked to select the "correct" stimulus from a choice of two pictures, with no instructions given as to how to make that selection. Following each response, subjects were given either positive or negative feedback, winning or losing money, with an 80:20 win/loss ratio for choosing the correct stimulus (and 100% loss for choosing the incorrect stimulus). The winning stimulus remained the same for 6-10 consecutive trials until a "reversal," at which time the other stimulus became the correct choice. Win and loss trials were contrasted with a neutral baseline using two different stimuli, one of which was identified to the participants as always correct, but involved no loss or gain of money. fMRI data were analyzed using the FreeSurfer Functional Analysis Stream (FS-FAST) version 5.3. Results: Behaviorally, there was a Group by Gender interaction in the number of correct responses: Alcoholic women made significantly more correct, rewarded responses than control women, whereas alcoholic men tended to choose the rewarded stimulus less frequently than control men. Both alcoholic men and women had less activation than their respective controls in the ventral striatum during rewarded trials. Alcoholic women had greater activation than control women in the posterior cerebellum during rewarded trials, whereas control men had greater activity than alcoholic men in this region. Conclusions: Alcoholic men showed an impairment in response bias toward the reinforced stimulus and had an associated hypoactivation of the ventral striatum and posterior cerebellum during reward. Alcoholic women's ability to select the rewarded stimulus was above that of the nonalcoholic control women in spite of striatal hypoactivation, suggesting a

compensatory shift in reward-based activation from the ventral striatum to the cerebellum, and corroborating reports of cerebellar involvement in reward-based learning.

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Poster

430. Cocaine Reinforcement II

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NIDA Grant T32 DA7288

Title: Transient optogenetic inhibition bidirectionally influences dendritic morphology in the nucleus accumbens core while attenuating cocaine-seeking behavior

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Abstract: Evidence suggests certain morphological and physiological changes in the nucleus accumbens NAc are important mediators of relapse. We previously demonstrated optogenetic inhibition of the NAc core subcompartment (NAcore) or its afferents from the prelimbic prefrontal cortex (PL) over a 2-hour reinstatement session attenuated cocaine seeking. Recent work suggests dynamic changes in plasticity occur during the first 15 minutes of these sessions, and these adaptations are blocked via pharmacological inhibition of the PL. Current work examined the behavioral and morphological consequences of optically silencing the NAcore, or its afferents in the PL during this first 15 minutes of cue-induced reinstatement. Male Sprague-Dawley rats underwent surgeries for microinjections of adeno-associated virus (AAV) coding for archaerhodopsin (ArchT) in either the PL or NAcore, implantation of bilateral fiber optics aimed the NAcore, and intra-jugular venous catheters. Studies were designed to assess selective inhibition of either PL terminals or cell bodies within the NAcore. Animals then went through 12 days of cocaine self-administration (FR1) followed by extinction training (2 hr/day). Following extinction, animals underwent cue-induced reinstatement along with the presence/absence of optical inhibition. Inhibiting PL-to-NAcore projections during the first 15 minutes sufficiently

blocked reinstatement behavior while the laser was on and paralleled a decrease in dendritic spine head diameter relative to controls. In animals receiving NAcore cell body inhibition, behavior was attenuated while the light was being delivered, but lever pressing rebounded when optical inhibition was relieved, and spine head diameter was potentiated. Electrophysiological experiments examined physiological changes resulting from optical inhibition. Results reveal interesting differences between reinstated behavior and spine morphology, suggestive that changes in spine morphology are a requisite antecedent to behavior.

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Poster

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NIH/NIDA P5012756

Title: In the blink of an eye: 500 msec cocaine cues trigger widespread activation of mesolimbic circuitry

Authors: ***A. R. CHILDRESS**[1,2], K. JAGANNATHAN[1], J. J. SUH[1,2], K. YOUNG[1], R. N. EHRMAN[1,2], Z. WANG[1], Z. MONGE[1], J. F. MAGLAND[1], T. R. FRANKLIN[1], R. R. WETHERILL[1], D. D. LANGLEBEN[1], M. GAWRYISAK[1,2], R. SZUCS-REED[1], C. P. O'BRIEN[1,2]
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Abstract: Aims: We humans are exquisite reward detectors. Honed by eons of fierce competition for food and sex, the sensitivity to reward signals ensures our very survival. Across millions of years, individuals who responded rapidly to the signals for (food or sexual) reward – even brief signals -- would have a reproductive advantage. Ironically, our highly-conserved, survival-driven sensitivity to reward signals has a potential dark side: individuals who struggle with addiction may be vulnerable even to very brief signals for drug reward. In a new cocaine

patient cohort, we tested whether very brief (500 msec) cocaine cues could trigger mesolimbic motivational circuitry. **Methods:** In a "fast" event-related BOLD fMRI paradigm, cocaine patients (n=27, ongoing) were exposed to cocaine-related and to comparison (sexual, aversive and neutral) cues of 500 msec duration; 48 images of each cue type were presented in quasi-random order. Image preprocessing (alignment, registration, normalization, smoothing, and motion correction) was conducted within a standard SPM 8 pipeline. Pre-planned contrasts (e.g., drug-neutral; sex-neutral, aversive-neutral) were based on the first 24 cue presentations of each cue type (minimizing the contribution of arousal "carry-over" in the second half of the task). **Results:** As predicted, cocaine patients evidenced a robust response to the brief 500 msec cocaine cues ($2<t<6$, $p<0.005$) in mesolimbic regions of interest, including the ventral tegmental area (VTA), bilateral amygdala, and bilateral ventral striatum/pallidum, as well as visual and modulatory (lateral orbitofrontal cortex, inferior frontal and anterior cingulate) regions. **Conclusions:** From an evolutionary perspective, a heightened sensitivity to rewards is a good thing, and would confer reproductive advantage. Drugs of abuse offer no reproductive advantage, but the brain treats signals for drug rewards as if they have true biologic value. The current data highlight the power of these brief signals, showing that cues occurring "in the blink of an eye" can have a profound impact on motivational circuits in the brain. Ongoing studies suggest the heightened response to brief cocaine cues in motivational/reward circuits is linked to relapse, underscoring the utility of the brief cue paradigm both as a tool for screening anti-relapse medications, and for identifying the "cue-vulnerable" patients who need these medications

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Poster

430. Cocaine Reinforcement II

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Title: Individual differences in motivation for cocaine assessed using a behavioral economics procedure

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Abstract: In humans, only a small proportion of the population that use drugs eventually become addicted. Among other symptoms, drug addiction can be characterized by 1) increases in time and resources spent seeking the drug, 2) an extremely high motivation to take the drug, and 3) continued drug use despite its known adverse consequences. Previous research has shown that only a limited proportion of rats trained to self-administer drugs develop all three of these addiction hallmarks. The goal of this research is to identify behavioral traits that may indicate an increased susceptibility to drug addiction and to develop more informative procedures to test for these symptoms of addiction. To this end, rats were initially screened for their propensity to attribute incentive salience to a discrete cue that was paired with a food reward in a Pavlovian setting (i.e., classed as sign-trackers [STs] or goal-trackers [GTs]). Rats were then trained to self-administer cocaine using a procedure that assured all rats had equal cocaine exposure. Following acquisition of self-administration, rats were then tested for their motivation to take cocaine and their willingness to take cocaine in the face of adverse consequences. To test the rats' motivation to take cocaine, a PMax procedure was used. In the PMax procedure, rats were allowed to nose-poke for cocaine on an FR-1 schedule with no timeout but the dose of cocaine that the rats received decreased every ten minutes. To test the rats' willingness to take cocaine in the face of adverse consequences we used a similar procedure, except here the dose of cocaine was held constant but the intensity of a shock paired with the drug infusion was increased every ten minutes. Following these baseline tests, rats were given 16 days of self-administration, but now using an intermittent access procedure. The intermittent access procedure consisted of alternating 5 minute blocks of cocaine access and 25 minute timeout blocks. The timeout blocks were signaled by the illumination of a red house light that was turned off during the drug available blocks. This procedure produces successive "spikes" in brain cocaine concentrations. In an initial study, we compared performance of STs and GTs between the first and second pMax and aversion probe tests, to assess the influence of experience with intermittent access to cocaine on subsequent motivation to take cocaine in STs and GTs. The studies are ongoing and the data will be presented at the meeting.

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Poster

430. Cocaine Reinforcement II

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NIH/NIDA (P5012756; T32DA028874)

VA VISN 4 MIRECC

Title: Your attention, please! The direction of brain-attentional bias correlations for cocaine cues depends on which hand generates the bias scores

Authors: Z. A. MONGE[1], J. J. SUH[1,2], R. EHRMAN[1,2], K. JAGANNATHAN[1], Z. WANG[1], J. F. MAGLAND[1], T. R. FRANKLIN[1], R. R. WETHERILL[1], K. A. YOUNG[1], M. GAWRYSIAK[1], D. D. LANGLEBEN[1], *C. P. O'BRIEN[3,2], A. R. CHILDRESS[1,2]
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Abstract: For addicted individuals, cues (e.g., sights, smells, sounds) that signal a rewarding drug may both attract ("Wow- cocaine!") and repel ("I shouldn't look at that - cocaine is trouble for me!") attention. We tested whether the early brain response to cocaine cues could predict their future "attention-attracting" vs. "attention-repelling" properties, using a dot-probe task of attentional bias that requires two hands for button pressing. Intriguingly, the brain-attentional bias correlations were in opposite directions for the two hands. Cocaine-addicted inpatients (n=25, ongoing) were scanned with event-related BOLD fMRI during exposure to brief (500 msec) evocative (cocaine, sexual, aversive) vs. neutral cues. Participants subsequently completed a visual "dot-probe" attentional bias task, designed to measure differential attention to cocaine vs. neutral cues. In this task, a positive attentional bias for cocaine was reflected in faster response (reaction time, RT) to an asterisk ("dot") when it occurred on the same side of the screen as a *prior* cocaine (vs. neutral) picture ($RT_{neutral}$ - $RT_{cocaine}$). Imaging preprocessing and first-level analysis was conducted within a standard SPM 8 pipeline. The attentional bias scores (msec; calculated separately for left-hand and right-hand responding) were then used as single regressors in a pre-planned (cocaine1-neutral1) cue contrast. Interestingly, the direction of the brain-attentional bias correlations depended entirely on the hand generating the bias scores. For trials where the cocaine cue was presented on the left (left hand responding), the brain-attentional bias correlations were *positive* in two reward-relevant nodes (putamen and OFC; $p<0.05$, uncorrected). In contrast, for trials where the cocaine cues were presented on the right (right hand responding), brain-attentional bias correlations were *negative* in several reward-relevant nodes (amygdala, hippocampus, OFC, ventral striatum, and VTA; $p<0.05$, uncorrected). This is the first report, to our knowledge, showing that the direction of the brain-attentional bias

correlations to evocative cues (in our case, cocaine cues) can depend on which hand generates the bias scores. This pattern suggests the right and left hemisphere may have distinct roles in processing the "attention attracting" vs. "attention-repelling " properties of stimuli, including drug cues. Clinically, novel treatment interventions (e.g., transcranial magnetic stimulation) might target only one hemisphere, with the goal of selective impact on the "attention-attracting" or "attention-repelling" properties of problematic cues.

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Poster

430. Cocaine Reinforcement II

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Research and Education Component of the Advancing a Healthier Wisconsin Endowment to CJ Hillard at the Medical College of Wisconsin.

Title: Relationship between stress-potentiated reinstatement of cocaine seeking and prefrontal cortical endocannabinoid signaling

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Abstract: In cocaine addicts, encounters with stressors confer vulnerability to relapse. Stimuli that do not ordinarily induce relapse often effectively do so in the context of stress. In order to better understand the underlying cellular mechanisms, we have developed an animal model termed stress-potentiated reinstatement wherein this phenomenon can be studied. In this model, rats acutely pre-exposed to a stressor, electric footshock (0.5 mA, 3x 0.2" duration; avg. 40" ITI over a 15-min period), reinstate cocaine seeking in response to an ordinarily sub-threshold dose

of cocaine (2.5 mg/kg, ip). Neither the footshock nor the sub-threshold cocaine alone induces reinstatement, but robust cocaine seeking is observed when the stimuli are combined. We have found that this phenomenon is corticosterone-dependent, as stress-potentiated reinstatement is not observed in adrenalectomized rats, and can be reproduced upon administration of corticosterone at a dose that establishes blood level in the stress-induced range. We have localized the effects of corticosterone to the prelimbic (PL) prefrontal cortex (PFC), an area reported to play a pivotal role in the reinstatement of drug-seeking behavior. As corticosterone has been shown to regulate endocannabinoid (eCB) signaling within the PL cortex, we hypothesized that eCB signaling in the PL PFC mediates stress-potentiated reinstatement. Consistent with this hypothesis, bilateral administration of the cannabinoid type-1 receptor (CB1R) antagonist, AM-251 (300 ng/side), into the PL cortex blocked stress-potentiated reinstatement. To examine stress-induced eCB mobilization in the PFC, we subjected drug-naïve animals to the same stress conditions that potentiate reinstatement, and then analyzed eCB concentrations via liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS; Agilent LC-MSD 1100 series) in the anterior cingulate, PL, and infralimbic PFC. Preliminary data suggest that footshock stress differentially increases levels of the eCBs, anandamide and/or 2-arachidonylglycerol, within these cortical subregions. In drug-naïve animals, footshock produced a significant increase of 2-arachidonylglycerol content within the anterior cingulate, while increased anandamide was found within the PL cortex. We will extend these findings to identify alterations in PFC eCB content that are associated with stress-potentiated reinstatement. Our data suggest that a key mechanism by which stress confers vulnerability to set the stage for relapse is through mobilization of eCBs within the PFC.

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Poster

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Title: Glucocorticoid-endocannabinoid interactions in the prelimbic cortex mediate stress-potentiated reinstatement of cocaine seeking

Authors: *J. R. MCREYNOLDS[1], E. M. DONCHECK[1], O. VRANJKOVIC[1], E. N. GRAF[1], C. J. HILLARD[2], J. R. MANTSCH[1]
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Abstract: Stress is a powerful trigger for relapse as it is unavoidable in daily life and can not only induce relapse/reinstatement but can potentiate the response to other triggers for drug use. We have previously shown that under certain self-administration conditions, stress alone does not reinstate cocaine seeking. However a stressor, such as electric footshock stress (EFS), can potentiate reinstatement when paired with a low dose of cocaine. This effect is corticosterone-dependent and the effect of EFS can be mimicked by systemic corticosterone administration suggesting that corticosterone is not only necessary but is also sufficient for this effect. Exactly how corticosterone potentiates reinstatement is not fully understood but may involve an interaction with the endocannabinoid (eCB) system. Stress increases eCB production in the medial prefrontal cortex (mPFC), a region critical for reinstatement, and, like stress-potentiated reinstatement, is glucocorticoid-dependent. Furthermore, corticosterone effects on mPFC neurotransmission are cannabinoid receptor 1 (CB1R) dependent. The present study examined the role of the CB1R, specifically in the prelimbic cortex (PL), in stress-potentiated reinstatement of cocaine seeking. Male SD rats self-administered cocaine under short-access conditions (0.5 mg/kg/infusion; 2 hr/day) and then underwent extinction training followed by stress-potentiated reinstatement tests. EFS paired with low dose cocaine (2.5 mg/kg, ip) induced reinstatement of cocaine seeking whereas either low dose cocaine or EFS alone did not. To test for the involvement of the PL in potentiated reinstatement, rats were given an intra-PL infusion of corticosterone (0.05 μg/.03 μL) instead of EFS 15 min prior to low dose cocaine. Intra-PL infusions of corticosterone mimicked the effect of EFS suggesting that corticosterone actions in the PL is sufficient to potentiate reinstatement. To test for the involvement of the CB1R, the CB1R antagonist, AM251 (0, 1, 3 mg/kg ip) was given 30 min prior to reinstatement tests. Systemic AM251 blocked stress-potentiated reinstatement suggesting a role for the eCB system. Finally, to examine the role of the CB1R in the PL, an intra-PL infusion of AM251 (0, 0.3 μg/0.3μL) was given 15 min prior to reinstatement tests. CB1R antagonism was tested with both EFS- and corticosterone-potentiated reinstatement. Preliminary data suggests that intra-PL AM251 blocks EFS- and corticosterone-potentiated reinstatement. These findings support the hypothesis that corticosterone actions in the PL, likely through an interaction with the eCB system, mediate stress-potentiated reinstatement of cocaine seeking.

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Poster

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MH-095972

Title: Cocaine self-administration in rats induces regressive structural plasticity in the medial prefontal cortex

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Abstract: The rodent medial prefrontal cortex (mPFC) is a critical component of a final common pathway that drives relapse of drug-seeking behavior, while other evidence indicates that drug addicts show inhibitory control deficits and hypoactivity in a medial prefrontal network under baseline conditions. Optimal functioning of mPFC relies on synaptic connections made onto dendritic spines in pyramidal neurons, and prefrontal dysfunction resulting from stress, aging, and mental illness are each linked to a decrease in dendritic spine plasticity in this cortical region. Paradoxically, the existing literature suggests that prolonged cocaine administration increases dendritic branching and spine density in the prelimbic cortical subfield (PL) of mPFC, raising questions as to how these structural alterations may translate to understanding addiction-related mental disorders. Here we critically examine the effects of prolonged cocaine administration on structural plasticity in PL using a high-throughout 3D spine morphometric analysis. Male Sprague-Dawley rats underwent surgery for implantation of intravenous jugular catheters and then underwent 2 weeks of cocaine self-administration (2 h daily) or served as yoked-saline or yoked-cocaine control rats. After two weeks of home cage withdrawal, animals were perfused and PL neurons were selected for intracellular injection of fluorescent dye, followed by 3D imaging and analysis of dendritic arborization patterns and spine morphometry. Whereas previous reports describe increases in dendritic branching and spine density in PL neurons following repeated cocaine administration, we failed to observe any increase in these indices as a function of any cocaine treatment group. Rather, rats that self-administered cocaine showed significantly decreased apical dendritic length (by 16%) and spine density (by 13%) in PL neurons compared to yoked-saline animals ($p < 0.05$ for each), and yoked-cocaine animals

failed to show any deficits in structural plasticity relative to the yoked-saline group. Animals that self-administered cocaine also displayed adrenal hypertrophy and reduced body weight gain relative to both yoked-cocaine and saline groups, effects typically found in animals that have experienced chronic stress. These data extend work from other laboratories suggesting that cocaine use, and not the effect of cocaine per se, represents a stressful experience for the individual. Moreover, the findings raise the possibility that the stress associated with drug self-administration may be a mechanism for inducing persistent changes in prefrontal plasticity that contributes to the addiction process.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Role of a CRF receptor-regulated dopaminergic projection from the VTA to the prelimbic cortex in stress-induced relapse

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Abstract: The neurobiological mechanisms and pathways through which stressful stimuli promote relapse to cocaine use remain elusive. Further research into these processes should guide the development of new and more effective approaches for managing cocaine addiction. We and others have implicated the neuropeptide, corticotropin releasing factor (CRF), in stress-induced relapse. In particular, we have shown that a CRF-releasing pathway that originates from the ventral bed nucleus of the stria terminalis (vBNST) and regulates the ventral tegmental area (VTA) is necessary for stress-induced cocaine seeking following long-access (LgA; 6h/day) cocaine self-administration (SA). The objective of this study is to examine whether CRF-mediated activation of mesocortical dopamine (DA) projections to the prelimbic cortex (PLC) is also necessary for stress-induced cocaine seeking. Specifically, we hypothesize that CRF release

into the VTA during stress activates a subset of VTA DA neurons that project to the PLC, resulting in DA D1 receptor activation and, thereby, reinstatement of drug-seeking behavior. To assess the activation of downstream VTA targets associated with stress-induced relapse, we first used immunohistochemistry to examine Fos expression in the PLC and other prefrontal cortical subregions in rats that had undergone LgA cocaine SA and extinction and were tested for reinstatement in response to a stressor, electric footshock (EFS), or under EFS-free control conditions. Preliminary data show that EFS increases Fos immunoreactivity in the PLC. Ongoing experiments using rats injected bilaterally with the retrograde tracer , cholera subunit B (CTb), into the PLC prior to reinstatement testing examine the relationship between stress-induced reinstatement and activation of DA cells in the VTA that project to the PLC, as determined using Fos and CTb co-immunoreactivity. The role of VTA CRF receptor-mediated activation of DA projections to the PLC and, thereby, D1 receptor activation in the PLC, in stress-induced reinstatement was examined using a disconnection approach involving unilateral intra-VTA delivery of the CRF-R1 antagonist, antalarmin (250ng) and contralateral intra-PLC administration of the D1 receptor antagonist, SCH 23390 (200ng). Disconnection of the PLC-VTA pathway blocked stress-induced reinstatement of cocaine seeking. Examination of the effects of ipsilateral antagonist delivery into the VTA/PLC is in progress. These preliminary findings suggest that CRF receptor activation in the VTA induces relapse to cocaine use by activating DA cells that project to the PLC.

Disclosures: O. Vranjkovic: None. **J.M. Blacktop:** None. **J.M. Resch:** None. **T. Kloehn:** None. **S. Choi:** None. **D.A. Baker:** None. **J.R. Mantsch:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: Opposing effects of pre- and postsynaptic adenosine A2A receptor blockade on cocaine seeking

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Abstract: Drug-associated cues or pharmacological stimuli are known to mediate cocaine seeking through enhanced dopamine and glutamate neurotransmission in the nucleus accumbens

(NAc). Previous work has shown that adenosine receptors modulate reinstatement to cocaine seeking as well as other cocaine-mediated behaviors. Adenosine A2A receptors are expressed on both pre- and postsynaptic terminals in the NAc. Postsynaptic A2A receptors in the NAc are colocalized on dopamine D2 expressing medium spiny GABAergic neurons where they reduce dopamine neurotransmission. Presynaptic A2A receptors are expressed on glutamate terminals in the NAc where they enhance glutamate transmission onto MSNs. The goal of these studies was to examine the differential effects of pre- and postsynaptic A2A receptor blockade on cocaine seeking. Rats were trained to lever press for cocaine in daily self-administration sessions on a fixed-ratio 1 schedule for 10 consecutive days. After one day of abstinence, lever pressing was extinguished in 8-10 daily extinction sessions. We subsequently identified whether a systemic administration of a presynaptic A2A receptor antagonist, SCH 442416, and a postsynaptic A2A receptor antagonist, KW 6002, would reinstate cocaine seeking. Higher doses of KW 6002 (0.3, 1.0, 3.0 mg/kg, i.p.) induced cocaine seeking, while SCH 442416 (1.0, 3.0 mg/kg, i.p.) had no effect. We next examined the effect of pre- and postsynaptic A2A receptor blockade on cocaine-induced reinstatement. Systemic administration of cocaine (15 mg/kg, i.p.) produced robust reinstatement that was blunted by pretreatment with the presynaptic A2A antagonist, SCH 442416 (1.0 mg/kg, i.p.). On the other hand, blockade of postsynaptic A2A receptors by KW 6002 (0.3 mg/kg, i.p.) amplified cocaine-induced reinstatement (5 and 15 mg/kg, i.p.). We hypothesize that SCH 442416 reduces reinstatement by dampening excessive cocaine-induced glutamate release in the NAc from the prefrontal cortex (PFC) that is necessary for cocaine seeking. In order to assess this hypothesis, cocaine seeking was induced by either an intra-PFC injection of AMPA (0.8 nM/side) or cocaine (200 μg/side). A systemic pretreatment with the presynaptic A2A antagonist, SCH 442416 (1.0 mg/kg, i.p.) significantly reduced both intra-PFC AMPA and cocaine induced reinstatement. These findings suggest that presynaptic A2A receptors may be a viable target in tempering the augmented glutamate release that plays a key role in driving reinstatement.

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Poster

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Title: CRF acts in the VTA to reduce NAc dopamine tone and promote cocaine seeking

Authors: *M. A. ROBBLE, R. C. TWINING, C. CHAN, A. L. EBBEN, A. J. JACOBSEN, D. S. WHEELER, J. R. MANTSCH, R. A. WHEELER
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Abstract: Stressful and aversive events promote maladaptive behaviors such as drug relapse by acting, in part, on the mesolimbic dopamine system. While the effects of aversive stimuli on dopamine neuron firing rates are mixed, studies of terminal dopamine release in the nucleus accumbens, commensurate with the experience of the aversive stimulus, indicate that such stimuli decrease dopamine signaling. Although it is unclear how aversive stimuli that cause reductions in terminal dopamine release engender motivated behavior, recent data suggest that the mechanism involves the modulatory actions of the stress-sensitive neuropeptide, CRF, in the VTA. Previous studies have shown that CRFR1 activation in the VTA is necessary for footshock-induced reinstatement, and that activation of CRFR1 in the VTA alone is sufficient to reinstate drug seeking. However, recent reports indicate that the role of CRF in modulating dopamine neuron activity in response to aversive stimuli can be altered by cocaine experience, raising additional questions about this mechanism of motivational regulation. We began to address these questions in a series of experiments. First, using fast-scan cyclic voltammetry, we determined that a primary aversive stimulus that decreases dopamine is sufficient to promote drug-seeking. In this design, rats were trained to self-administer cocaine on an escalating regimen. Once intake reached criterion, rats were transitioned to extinction training in which the operant response no longer yielded the programmed consequence of cocaine administration. In extinction training, rats reduced responding to a low, stable baseline. Then rats were given a test session in which the aversive tastant, quinine, was intra-orally administered to the rat prior to the extinction session (1inf/min, 200ul/inf, 15min). Compared to the previous day of responding, quinine administration significantly increased responses on the lever that previously provided cocaine. In a subsequent study we found that this effect is dependent on the actions of CRF, as reinstatement was blocked by bilateral intra-VTA infusions of CP-376395, a CRFR1 antagonist. Consistent with this, we used fast-scan cyclic voltammetry to determine that the ability of quinine to reduce NAc dopamine tone also was blocked by the bilateral intra-VTA infusion of the CRFR1 antagonist in naïve animals. Finally, we verified that quinine both reduced dopamine signaling and induced aversive taste reactivity in naïve and cocaine-experienced animals. Current studies are examining the possibility that the CRF regulation of terminal dopamine concentration is altered in cocaine experienced animals.

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Poster

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Title: Development of an animal model of comorbid PTSD and cocaine addiction

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Abstract: Substance use disorders (SUDs) and post-traumatic stress disorder (PTSD) are at crisis levels in the United States military. In the general population as well in veterans, there is a high co-occurrence of PTSD and SUD. Patients who display comorbidity for these disorders are more resistant to treatment. An animal model for comorbid PTSD and stimulant addiction is greatly needed in order to screen medications for their ability to reduce symptoms of both disorders. Here we compare two methods of inducing PTSD-like symptoms in Sprague-Dawley rats. Rats are exposed to one of two types of predator stress (cat urine or TMT, a compound found in fox urine) once for 10 minutes. One week later, rats are tested in the elevated-plus maze (EPM) and acoustic startle boxes. Rats are classified as having a PTSD-like phenotype or non-PTSD phenotype based on the percent time spent in the open arms of the EPM and the degree of habituation of the acoustic startle response. Subsequently, rats self-administered cocaine for one hour per day for seven days followed by ten days of 6-hour daily access. Animals went through extinction training and were tested for the reinstatement of cocaine-seeking. We found that one 10-minute exposure to TMT is sufficient to induce PTSD-like symptoms in 20% of rats, evidenced by significant reduction in time spent in the elevated plus maze and a failure to habituate the startle response. A single exposure to cat urine resulted in a similar effect on the startle response but did not behavior on the EPM. Neither TMT nor cat urine significantly altered the amount of cocaine self-administered. However, rats exposed to TMT and classified as having a PTSD-like phenotype demonstrated a strong trend towards enhanced cue-primed reinstatement relative to control rats and TMT-exposed rats that did not develop a PTSD-like phenotype. Re-exposure to the context in which predator stress was experienced produced a significant suppression of reinstatement behavior when animals were tested immediately after exposure and no change in behavior when they were tested 48 hours after re-exposure to the context. These results provide support for predator stress as a model of PTSD in rats. Neurobiological substrates of comorbid PTSD and SUD vulnerability will be investigated in future studies.

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Title: Metabolic changes in rat brain following cocaine self-administration

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Abstract: Cocaine dependence is a public health issue and the cause of significant morbidity and mortality globally. This powerful-addictive psychostimulant when chronically used can induce stable and long-lasting neurochemical effects within different brain regions. At the present time, there are no effective pharmacotherapies available that can cure cocaine addiction. Therefore, understanding the neurobiology of cocaine dependence is still a research priority for future pharmacological treatments. Analyzing the complete pool of metabolites in an organism seems to be a promising approach for understanding biochemical pathways affected by addiction. Our experimental goal is to generate and compare untargeted metabolomic profiles of addictive and healthy states using specific brain regions related to cocaine dependence. This approach will allow us to detect affected metabolic pathways, understand drug addictive behaviors and identify diagnostic biomarkers associated with this illness. In all studies, male Sprague-Dawley rats were trained to self-administer cocaine in a cue-elicited drug seeking behavior paradigm. Brain dissection of the prefrontal cortex (PFC), hippocampus, cerebellum and nucleus accumbens of both control and experimental rats were performed for the extraction, derivatization and analysis of metabolites via nontargeted metabolomics using gas chromatography-mass spectrometry. We identified 31 metabolites in the PFC, 17 in the hippocampus and 43 in the cerebellum. Bioinformatics analysis was done using Mataboanalyst.ca web based resource including Partial least squares Discriminant Analysis (PLS-DA) and Metabolite Enrichment Analysis (MAE). PLS-DA analysis revealed that self-administration of cocaine produced significant metabolic changes in the cerebellum and PFC compare to the hippocampus. As identified by MEA, the metabolic pathways affected in the cerebellum were amino acid metabolism, protein synthesis

and glucose-alanine cycle while in the PFC significant changes were found in fatty acid metabolism, citric cycle, insulin signaling, glycerolipid metabolism and amino acid metabolism as well. Taken together our data indicate that cocaine addiction affects diverse metabolic pathways, which are specific for each functionally specialized brain region.

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Poster

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DA034684

Title: A history of repeated sodium depletions in rats alters the reinforcing properties of cocaine assessed during self-administration

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Abstract: The experience of sodium depletion engages neural circuitry critical for the performance of motivated behaviors in order to promote the seeking and ingestion of sodium. Sodium depletion also induces neuroplasticity in motivation and reward neural circuitry. This neuroplasticity may have at least two long-term effects on behavior: altered sensitivity to addictive drugs and enhanced sodium intake. Preclinical work has demonstrated a link between sensitivity to drugs of abuse and sodium appetite. Specifically, rats with a history of cocaine injections exhibit enhanced salt intake and rats with a history of sodium depletions exhibit enhanced cocaine-induced locomotor activity. The present experiments were conducted to determine whether repeated episodes of sodium depletion would affect cocaine self-

administration and to determine how sodium depletion may alter motivation and reward systems. In the first experiment rats received either 4 sham or 4 sodium depletions and were implanted with jugular catheters. Self-administration for cocaine was tested at a descending range of doses (90, 30, 9, 3 μg/infusion). The results indicated a significant interaction between dose and group which suggested a rightward shift in the dose-response curve for the sodium-depleted rats. The second experiment determined how brain areas involved in sensing sodium deficiency may influence activity of motivation and reward systems. The lateral hypothalamus (LH) was a candidate region as it has been implicated in motivated behavior and contains dense projections to the ventral tegmental area (VTA). The retrograde tracer fluorogold was iontophoresed into the LH and retrograde labeling was observed across structures along the lamina terminalis (LT), an ensemble of forebrain nuclei important for sensing sodium status. A third experiment was conducted to investigate whether neuroplasticity in the VTA is critical for the enhanced sodium intake seen after sodium depletion. The NMDA receptor antagonist AP-5 was microinjected into the VTA prior to an acute sodium depletion and 4 days later rats received a second sodium depletion. Vehicle pretreated rats exhibited an increase in sodium intake, while AP-5 pretreated rats failed to elevate sodium intake. These experiments suggest that prior sodium depletions alter the reinforcing properties of cocaine, as assessed with self-administration. Additionally, sodium deficiency is sensed by the LT and this information may be passed on to the LH and in turn to the VTA. Neuroplasticity in the VTA serves to moderate sensitization of increased salt intake and is most likely also responsible for altered cocaine self-administration.

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Support: P01 DA 08227

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Title: Enhancement of cocaine reinforcement by selective loss of TrkB-PLCγ, but not TrkB-ERK, signaling in the nucleus accumbens shell

Authors: *E. M. ANDERSON, D. GUZMAN, A. M. WISSMAN, D. W. SELF
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Abstract: Previous work indicates that BDNF signaling through TrkB receptors in the nucleus accumbens shell (NACsh) enhances cocaine reinforcement. However, the TrkB signaling pathways responsible for this effect are unknown. Distinct TrkB tyrosine residues can activate either ERK or PLCγ, and we hypothesized that selective impairment through either pathway would determine their necessary role in BDNF-TrkB effects on cocaine reinforcement. Novel HSV vectors were constructed including a kinase-dead dominant negative TrkB mutant that attenuates global TrkB function (HSV-TrkB-KD). Other vectors contained a point mutation that either selectively blocks TrkB-ERK signaling but preserves TrkB-PLCγ signaling (HSV-TrkB-ERK), or vice versa (HSV-TrkB-PLC), and a wild-type TrkB (HSV-TrkB-WT) tested a gain of function. HSV-infected Hela cells were used to assess BDNF (50 ng/ml) activation of phosphorylation targets with western blots. Rats implanted with bilateral NACsh cannulae were trained to self-administer (SA) cocaine on fixed ratio (FR) responding for 3-4 weeks until stable. Following stability, rats were tested in a within-session dose-response for cocaine SA over 5 days before HSV infusions, 5 days during HSV-mediated expression, and for 5 days after HSV-mediated expression dissipates. BDNF-TrkB induced ERK phosphorylation was attenuated in Hela cells infected with HSV-TrkB-ERK, whereas PLCγ phosphorylation was attenuated by HSV-TrkB-PLC, and both pathways disrupted in cells infected with HSV-TrkB-KD compared to HSV-GFP-infected controls. HSV-TrkB-WT had no effect on the BDNF response. TrkB mutants produced prominent immunoreactivity for FLAG (TrkB) colocalizing with bicistronic GFP confined to the shell subregion 3 days following HSV infusions. In animals trained to SA cocaine, infusions of either HSV-TrkB-KD or HSV-TrkB-PLC exhibited a transient leftward shift in the dose threshold necessary to maintain cocaine SA behavior compared to HSV-GFP controls, indicating increased sensitivity to cocaine reinforcement with loss of endogenous TrkB-PLCγ signaling. In contrast, neither HSV-TrkB-ERK nor HSV-TrkB-WT altered cocaine SA, indicating that loss of endogenous TrkB-ERK signaling in NACsh neurons fails to alter sensitivity to cocaine reinforcement. Intact PLCγ signaling (TrkB-ERK mutant) rescues the dominant negative phenotype and addiction-promoting effects of TrkB-KD in NACsh neurons. The enhanced sensitivity to cocaine reinforcement with either global (TrkB-KD) or pathway-specific (TrkB-PLC) loss of TrkB function are paradoxical given that localized knockout of either BDNF or TrkB in NACsh attenuates cocaine reinforcement.

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Poster

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Title: Ceftriaxone requires both xCT and GLT-1 up-regulation in the nucleus accumbens to attenuate the reinstatement of cocaine-seeking

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Abstract: Ceftriaxone is a beta-lactam antibiotic which increases the expression and function of the glutamate transporter GLT-1 and of system xC-, which exchanges extracellular cysteine for intracellular glutamate. Basal glutamate levels in the nucleus accumbens are largely controlled by system xC-, and a decrease in its activity is a contributing cause of the altered glutamate homeostasis observed in this brain region following cocaine self-administration in rats. The catalytic subunit of xC- is xCT, and we have demonstrated that expression of xCT and GLT-1 is decreased in the nucleus accumbens core following cocaine self-administration. We have also shown that ceftriaxone attenuates cue- and cocaine-primed reinstatement while restoring levels of both xCT and GLT-1 in the nucleus accumbens core. At this time it is not known if an increase in both transport systems is essential for the attenuation of cocaine reinstatement by ceftriaxone. Here we used a morpholino antisense strategy to decrease the expression of xCT and GLT-1 protein and examined the relative importance of these two proteins in mediating the reinstatement of cocaine-seeking. Altered glutamate homeostasis in the accumbens has also been shown to change post-synaptic glutamate receptor function and expression. Thus we assessed the nucleus accumbens surface expression of AMPA receptor subunits and total protein expression of mGluR5 and the subunits of AMPA and NMDA receptors. Rats were trained to self-administer cocaine for two weeks in an operant chamber and then experienced extinction training for three weeks. Subsequently, rats were treated with systemic ceftriaxone (200 mg/kg IP) or vehicle and intra-accumbens active or control antisense morpholino. We found that rats treated with ceftriaxone in the presence of xCT and GLT-1 antisense did not show attenuated cue-primed reinstatement, indicating that both proteins are critical to the actions of ceftriaxone. We also found a trend for a reduction in surface GluR2 expression in cocaine animals that was reversed by ceftriaxone and not affected by xCT or GLT-1 knockdown. These data support the importance of increasing both GLT-1 and xCT expression in the attenuation of cocaine reinstatement.

Disclosures: J.L. Bilodeau: None. **L. Knackstedt:** None. **K. Reissner:** None.

Poster

430. Cocaine Reinforcement II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 430.16/DD11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: Conditioned and unconditioned Corticosterone elevations associated with cocaine self-administration

Authors: *Z.-B. YOU, R. A. WISE
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Abstract: Blood samples were taken and corticosterone (CORT) assayed at various stages of cocaine self-administration, extinction, and reinstatement experiments. CORT levels became elevated by 50% over the first two hours of self-administration and remained elevated for at least two hours after the end of four-hour sessions. CORT levels were elevated more modestly_by about 25%_in cocaine-naïve animals subjected to the same handling and cage transfer but allowed to self-administer only saline. Yoked cocaine injections caused a similar_25%_elevation in saline-trained rats. In cocaine-trained rats, however, CORT levels rose to twice baseline levels over the first 2h of yoked cocaine injections in cocaine-trained rats. Yoked injections of cocaine methiodide_cocaine MI, a cocaine analogue that shares the peripheral properties of cocaine but does not cross the blood-brain barrier had an even stronger effect over approximately the same time course. Cocaine MI had no effect on CORT levels in animals that had not previously been trained with “regular” cocaine (cocaine HCl), reflecting the conditioned association of the peripheral with the central effects of cocaine during self-administration training. CORT levels were elevated by about 75% during extinction training, confirming that cocaine expectancy was a factor in the elevated CORT levels. Following two weeks of extinction training, priming injections of cocaine HCl elevated CRF levels by 130%, whereas cocaine MI priming elevated CORT levels by about 40% and saline priming was ineffective. These data suggest that the stressful effects associated with cocaine self-administration reflect an interaction between cocaine expectancy and the pharmacological effects of the drug.

Disclosures: Z. You: None. **R.A. Wise:** None.

Poster

430. Cocaine Reinforcement II

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Program#/Poster#: 430.17/DD12

Topic: C.17. Drugs of Abuse and Addiction

Title: Methyl supplementation via L-Methionine attenuates addictive-like behaviors in rats and blocks c-Fos activation in the reward circuit following cocaine-primed reinstatement

Authors: *K. N. WRIGHT[1], F. HOLLIS[2], F. DUCLOT[1], D. M. DIETZ[3], T. C. FRANCIS[4], R. MERCER[1], A. M. DOSSAT[1], C. E. STRONG[1], J. FENG[5], M. LOBO[4], E. J. NESTLER[5], M. KABBAJ[1]
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Abstract: Epigenetics has recently come to light as a major contributing factor to the development of addiction as well as relapse, a major problem many individuals face on the path to recovery. DNA methylation, the induction of, in most cases, a transcriptionally repressive state via the catalysis of a methyl group onto cytosine bases by DNA methyltransferases (Dnmts), has been shown to play a role in learning and memory as well as conditioned place preference to cocaine, but its involvement in reinstatement is unknown. L-Methionine (MET) is a known methyl donor for Dnmts and has previously been shown to hypermethylate specific genes in rodent cortex and striatum upon supplementation. To investigate the effects of methyl supplementation on addictive-like behaviors, rats received chronic treatment of MET during drug-induced locomotor sensitization and intravenous cocaine self-administration. MET decreased behavioral sensitization to the locomotor-activating effects of cocaine and reduced drug-seeking behavior during cocaine-primed reinstatement. Furthermore, cocaine self-administration induced global DNA hypomethylation in the nucleus accumbens (NAc) after cocaine-primed reinstatement. While MET treatment did not alter global DNA methylation in either of the experimental groups, specific gene targets are likely as MET treatment blocked cocaine-induced c-Fos activation in both the NAc and the medial prefrontal cortex (mPFC). This suggests an important role for this circuit in cocaine-primed reinstatement that may be mediated by an increase in CpG methylation at the c-Fos promoter region. Finally, using an optogenetic approach, we are currently examining the role of mPFC-NAc communication on MET's effects on blocking cocaine-primed reinstatement.

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Poster

430. Cocaine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant 5R01DA025646-03

Title: Optogenetic inhibition of a lateral orbitofrontal to basolateral amygdala subcircuit impairs cue-induced cocaine-seeking behavior in rats

Authors: A. A. ARGUELLO[1], M. A. HODGES[2], G. D. STUBER[2], *R. A. FUCHS[1]
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Abstract: Cocaine addiction is a chronic relapsing disorder, characterized by compulsive drug-seeking and -taking behavior even after prolonged periods of abstinence. Relapse is a key problem in the successful treatment of cocaine addiction, complicated by the fact that it can be triggered by multiple drug-associated cues and recruits several brain circuits. We have previously shown that the lateral orbitofrontal cortex (lOFC) and basolateral amygdala (BLA) share dense reciprocal connections and control motivated behavior in response to drug-associated cues. Thus we hypothesized that a specific monosynaptic subcircuit that projects from the lOFC to the BLA mediates drug-associated explicit conditioned stimuli (CS)-induced cocaine-seeking behavior. To test this hypothesis, male Sprague-Dawley rats received bilateral infusions of a Cre-dependent adeno-associated viral (AAV) vector expressing halorhodopsin with a fluorescent reporter protein (DIO-eNpHR3.0-mCherry) or the fluorescent reporter protein alone (DIO-mCherry) as well as optic fiber placement into the lOFC. The same rats also received bilateral infusions of a retrogradely transported AAV vector expressing Cre recombinase (Cre-GFP) into the BLA, permitting selective recombination and expression of halorhodopsin in a subset of lOFC neurons that project to the BLA. Following recovery, rats were trained to lever press for cocaine infusions and simultaneous presentations of a CS complex (light + tone). Rats then underwent extinction training, during which active lever responses no longer resulted in cocaine infusion or CS presentation. At test, cocaine-seeking behavior (i.e., non-reinforced lever

presses) was assessed with response-contingent CS presentation coupled with laser stimulation (5 sec, 532 nm) to produce CS-coupled optical inhibition of halorhodopsin-expressing lOFC neurons that project to the BLA. Rats were tested in a counterbalanced fashion with or without laser stimulation at test. Optogenetic inhibition of the lOFC disrupted the ability of the CS to reinstate cocaine-seeking behavior relative to the responding observed without optogenetic inhibition and in DIO-mCherry controls that do not express halorhodopsin. These findings suggest that input from the lOFC to the BLA is necessary for CS-induced cocaine-seeking behavior.

Disclosures: A.A. Arguello: None. **G.D. Stuber:** None. **R.A. Fuchs:** None. **M.A. Hodges:** None.

Poster

430. Cocaine Reinforcement II

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Program#/Poster#: 430.19/DD14

Topic: C.17. Drugs of Abuse and Addiction

Support: PR-110146

Title: Exploring whether cocaine-induced priming of innate immune signaling is TLR4 mediated and contributes to sensitized dopamine responsiveness

Authors: *T. J. FABISIAK[1], A. L. NORTHCUTT[2], T. A. COCHRAN[2], M. D. WEBER[2], M. R. HUTCHINSON[3], S. F. MAIER[2], K. C. RICE[4], R. K. BACHTELL[2], L. R. WATKINS[2]
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Abstract: The initial reinforcing properties of drugs of abuse, such as cocaine, are largely attributed to their ability to activate the mesolimbic dopamine system. Resulting increases in extracellular dopamine in the nucleus accumbens are traditionally thought to arise from cocaine's ability to block dopamine transporters. Repeated cocaine use can lead to addiction, a disease state in the brain associated with neuroplasticity. The phenomenon of behavioral sensitization and augmented dopaminergic signaling in response to repeated exposure to cocaine is well documented and thought to underlie cocaine's addictive effects; however, through what mechanisms cocaine generates these changes is still not well-understood. However, we have

recently demonstrated that cocaine can also activate the immunosurveillance receptor complex, Toll-Like Receptor 4 (TLR4). TLR4 is located primarily on glial cells, and activation initiates CNS innate immune signaling, involving the release of proinflammatory substances. Disruption of cocaine signaling at TLR4 (1) prevents induction of CNS immune activation, (2) suppresses conditioned place preference, (3) blocks drug-induced dopamine increases within the nucleus accumbens and (4) attenuates drug self administration. These findings provide the foundation for our recently proposed xenobiotic hypothesis. This hypothesis suggests that in serving its immune-surveillance role, TLR4 detects and identifies morphine and cocaine as foreign, invading compounds and initiates proinflammatory immune signaling in response to the perceived threat. Further, we have shown that cocaine reward and reinforcement are TLR4-depedent. It is unknown what effect repeated cocaine exposure has in relation to TLR4 and CNS immune signaling. However, glial cells have an interesting propensity towards "priming", meaning that with repeated activation, the subsequent proinflammatory responses are increasingly robust. Here, using a rapid microglial isolation paradigm, we investigate the hypothesis that (1) repeated cocaine administration results in the development of a primed CNS innate immune response and (2) whether this effect is mediated by TLR4. Further, using *in vivo* microdialysis, we explore (3) the influence of cocaine-primed CNS immune signaling on the development of augmented dopamine release within the nucleus accumbens.

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Poster

430. Cocaine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R21-DA029787

VA Grant 589-KG-0012

Title: High-dose donepezil attenuates the subjective effects of intravenous cocaine in non-treatment seeking participants

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Abstract: Despite pronounced effects on drug-reinforced behavior in animals, cholinesterase inhibitors have had disappointing results on clinical measures in cocaine dependent humans. For example, previous evaluations of the acetylcholinesterase inhibit donepezil failed to diminish drug taking in treatment-seeking participants and augmented some subjective effects of cocaine in a laboratory setting. Because disappointing results of earlier patient-based studies may reflect the relatively low doses of donepezil utilized, we evaluated actions after rapid escalation to relatively high dose levels of donepezil. METHODS: A randomized, single-blind, placebo-controlled, single-center laboratory design was utilized. Non-treatment-seeking, regular cocaine users received either oral placebo or donepezil rapidly titrated to a final dose of 22.5 mg daily. In a laboratory setting, participants than received intravenous placebo or cocaine (0.23 and 0.46 mg/kg). Subjective effects of cocaine were than measured by visual analogue scale. RESULTS: A total of 11 participants were randomized to donepezil, with nine participants completing the full protocol and two subjects discontinuing early before the second intravenous dosing session because of personal reasons. Five donepezil-treated subjects reported mild to moderate gastrointestinal symptoms, which included abdominal discomfort, nausea, vomiting, or diarrhea. Two of these individuals required a reduction from 22.5 to 15.0 mg daily, but there were no discontinuations of active treatment because of adverse events. Aside from one syncopal event after combined treatment with donepezil and cocaine, the study medications were well tolerated. Active treatment with donepezil attenuated cocaine-induced ‘high’ and self-report of stimulation. When averaged over the 20 minutes following infusions, drug-induced 'high' was attenuated by greater than two- and four- fold, for 0.46 and 0.23 mg/kg of cocaine, respectively. CONCLUSION: Rapid titration to a final daily dose of 15.0 or 22.5 mg of donepezil daily over a period of 16 days was well tolerated. Previously evaluated doses have been based on the relatively slow escalation of donepezil dose in studies of patients with Alzheimer disease. When titrated to dose levels that more resemble those used in infrahumans, donepezil has greater utility in decreasing the positive subjective effects of cocaine. Nonetheless, cholinergic actions may limit its utility in treating cocaine-use disorders.

Disclosures: K.W. Grasing: None. **C. DeSouza:** None. **T. Newton:** None.

Poster

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Title: Relapse-suppression by drug omission cues

Authors: *N. SUTO[1], B. T. HOPE[2], M. MAYFORD[1], R. A. WISE[2], G. I. ELMER[3], F. WEISS[1]
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Abstract: Environmental cues signaling the availability of drugs of abuse are known to precipitate drug seeking and craving even following long periods of abstinence. In contrast to such relapse-promoting actions of drug availability cues, environmental cues signaling drug unavailability (here termed 'drug omission cues') may suppress relapse. To test this hypothesis, an 'omission cue-induced suppression (OCIS)' procedure was developed and examined in rats under experimental conditions linked to high relapse risk. Separate groups of rats underwent operant conditioning sessions to self-administer cocaine or alcohol. Each rat was then subjected to alternating sessions in which operant responding were reinforced on some ('drug sessions') but not others ('omission sessions'). The omission sessions were preceded and accompanied by a discriminative stimulus signaling the unavailability of the drug reward (S-). During the course of the OCIS procedure, the rats were subjected to a long period of cocaine intake or alcohol dependence (induced by alcohol liquid diet), each known to result in behavioral and brain changes in rats resembling those observed in addicts. In cocaine-trained rats, the S- signaling cocaine omission significantly suppressed drug seeking elicited by cocaine availability cues, stress, and cocaine priming. In alcohol dependent rats undergoing acute or protracted withdrawal, the S- signaling alcohol omission significantly suppressed drug seeking elicited by alcohol availability cues, stress, and alcohol priming. Thus, drug omission cues are capable of suppressing major modes of relapse-promotion across two classes of commonly abused drugs. The relapse-suppressing actions of omission cues are presumably mediated by learning processes known as conditioned inhibition or negative occasion setting, and distinct from extinction. Both cocaine omission cues and alcohol omission cues significantly increased Fos-positive ('activated') neurons within the infralimbic (IL) and prelimbic (PL) cortices of the medial prefrontal cortex, brain regions implicated in cognitive control of drug craving. Selective disruption of omission cue-activated neurons in the IL (achieved by the Daun02 method - a pharmacogenetic tool to induce apoptosis in Fos-positive neurons) blocked OCIS in an additional group of rats trained with a non-drug reward (glucose/saccharine). Further research of OCIS and

its neurobiological basis may guide the advancement of anti-relapse strategies by revealing neurobehavioral factors that actively suppress - rather than promote - drug seeking. **Support:** NIDA and NIAAA/NIH, DA033533 (N.S.), DA007348 (F.W.), and AA021549 (F.W.).

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Poster

430. Cocaine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA018832

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State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development

Title: Novel mixed-action mu-opioid receptor (mor) agonist/delta-opioid receptor (dor) antagonist that prevents stress-induced reinstatement of extinguished cocaine seeking behavior

Authors: ***K. A. HYMEL**[1], S. O. EANS[1], M. L. GANNO[1], E. MIZRACHI[1], N. ROSS[1,2], S. N. SENADHEERA[2], J. V. ALDRICH[2], J. P. MCLAUGHLIN[1]
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Abstract: Opioid ligands with mixed MOR agonist and DOR antagonist activity have shown promise as analgesics with reduced liabilities, and DOR antagonists have proven effective in reducing ethanol consumption. However, little is known about the effects of such compounds on cocaine seeking-behavior. We hypothesized that in addition to producing analgesia with fewer liabilities, an opioid ligand with MOR agonist and DOR antagonist activity would also prevent reinstatement of extinguished cocaine-seeking behavior. Accordingly, we tested two putative mixed opioid agonist/antagonists, (-)pentazocine and the novel cyclic peptide JVA-3025, in male C57BL/6J mice for opioid efficacy and selectivity in the 55oC warm-water tail-withdrawal assay, and then determined both compounds' effects on the reinstatement of extinguished cocaine-seeking behavior in a conditioned place preference (CPP) assay. (-)Pentazocine and JVA-3025 demonstrated full efficacy in the tail-withdrawal assay, with ED50 (and 95% CI)

values of 2.51(1.66-4.27) and 16.00(12.16-21.38) nmol, i.c.v., respectively. JVA-3025 also produced significant antinociception after oral administration. Whereas (-)pentazocine antinociception was mediated by all three opioid receptors, JVA-3025 antinociception appeared to be primarily MOR mediated. Pretreatment (>2 h, i.c.v.) with either JVA-3025 or (-)pentazocine selectively antagonized DOR antinociception. Repeated treatment with JVA-3025 and (-)pentazocine did not result in significant tolerance in an acute opioid antinociceptive tolerance model. In conditioned place preference testing, JVA-3025 resulted in no preference, whereas higher doses of (-)pentazocine demonstrated CPP. However, after i.c.v. pretreatment (>2 h), both compounds dose-dependently prevented stress-, but not cocaine-, induced reinstatement. These data support the theory that DOR antagonists may provide potential treatment for cocaine abuse, and suggest that with its distinct opioid activity profile, JVA-3025 is a promising compound for therapeutic development, both as a novel analgesic with reduced liabilities and to prevent stress-induced cocaine relapse. Funding provided by NIDA (DA018832 and DA032928) and the State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development.

Disclosures: **K.A. Hymel:** 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.; National Institute on Drug Abuse. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); State of Florida, Executive Office of the Governor's Office of Tourism, Trade, and Economic Development. **S.O. Eans:** None. **M.L. Ganno:** None. **E. Mizrachi:** None. **N. Ross:** None. **S.N. Senadheera:** None. **J.V. Aldrich:** None. **J.P. McLaughlin:** None.

Poster

430. Cocaine Reinforcement II

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Support: DA030676

DA013429

Title: Clavulanic acid and ceftriaxone enhance extinction of cocaine's reinforcing effects in rats: A new indication for a β-lactamase inhibitor?

Authors: *J. A. SCHROEDER[1], E. M. RIDENER[2], C. S. TALLARIDA[3], V. SVYSTUN[2], J. W. PICKEL[2], S. M. RAWLS[3]
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Abstract: Despite the efficacy of ceftriaxone (CTX) in animal models of CNS diseases, including drug addiction, its utility as a CNS-active therapeutic may be limited by poor brain penetrability and cumbersome parenteral administration. An alternative is the β-lactamase inhibitor clavulanic acid (CA), a constituent of Augmentin that prevents antibiotic degradation. CA possesses the β-lactam core necessary for CNS activity but, relative to CTX, possesses: 1) oral activity; 2) 2.5-fold greater brain penetrability; and 3) negligible antibiotic activity. To compare the effectiveness of CA (10 mg/kg) and CTX (200 mg/kg) against the rewarding effect of an abused drug, we investigated their effectiveness against conditioned place preference (CPP) produced by cocaine. Endpoints were based on prior evidence that CTX reduces reinforcing, seeking and locomotor-activating effects of cocaine. Results showed that rats treated with cocaine (10 mg/kg) displayed significant CPP compared to saline-treated controls (P 0.05). However, in the case in which CA or CTX was administered to rats that already developed CPP to cocaine, both drugs enhanced extinction of the CPP ($P < 0.05$). Ongoing experiments are investigating c-fos activity in the nucleus accumbens to link the behavioral outcomes to neurochemical changes. The present findings are the first to suggest that CA disrupts the *in vivo* actions of cocaine and point toward further studying CA as a potential therapy for drug addiction. Further, its ability to disrupt cocaine's rewarding effects at 20-fold lower doses than CTX identifies CA as an existing, orally-active alternative to direct CTX therapy for CNS diseases.

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Poster

430. Cocaine Reinforcement II

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Topic: C.17. Drugs of Abuse and Addiction

Title: Region and context specific intracellular responses associated with cocaine-induced conditioned place preference expression

Authors: *A. KLAMBATSEN[1,2], S. K. NYGARD[3,1,2], B. BALOUCHA[1], S. JENAB[1], V. QUINONES-JENAB[1]
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Abstract: The development and maintenance of cocaine addiction depend heavily on learned reward-environment associations that can induce drug-seeking behavior and relapse. Understanding the mechanisms underlying these cue-induced conditioned responses are important for relapse prevention. To test whether intracellular responses measured after cocaine conditioned place preference (CPP) expression are context-dependent, we re-exposed cocaine-treated rats to an environment previously paired with cocaine or saline, drug-free after 8 days of cocaine CPP training with one of two cocaine doses (5mg/kg or 20mg/kg, i.p). CPP was expressed only after conditioning with the higher dose of cocaine and locomotor responses after re-exposure to the cocaine-chamber were greater than in rats re-exposed to the saline-paired chamber. Nucleus Accumbens (NAc) phosphorylated ERK (pERK) levels were increased after re-exposure to the cocaine-paired, but not the saline-paired environment 24 hours after the CPP test, regardless of whether or not CPP behavior was expressed. Caudate Putamen (CPu) pERK and FosB protein levels increased after re-exposure to the cocaine chamber only after conditioning with the higher cocaine dose. Conversely, the higher cocaine dose, independent of environment, resulted in increased NAc FosB, ΔFosB and phosphorylated CREB (pCREB) protein levels compared to those conditioned with 5mg/kg cocaine (non-CPP-expressing). Our results suggest that NAc ERK phosphorylation may be involved with retrieving the contextual information of a cocaine-association, without necessarily motivating the expression of CPP behavior. Additionally, we show distinct patterns of intracellular responses in the NAc and CPu indicating a region specific role for pERK/pCREB/FosB intracellular signaling in the retrieval of cocaine-context associations.

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Poster

430. Cocaine Reinforcement II

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PA DOH

Title: Sleep regulates incubation of cocaine craving in rats

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Abstract: Both human and animal studies suggest that sleep quality deteriorates during long-term abstinence from repeated use of addictive drugs such as cocaine. This poor sleep quality has been speculated to contribute to relapse, mainly based on correlation analysis in humans and sleep deprivation studies in animals. However, sleep deprivation is not the same as insomnia - thus it remains unclear whether, and how, poor sleep consequent to drug use exacerbates addiction-related behaviors during abstinence; and whether interventions of sleep could alleviate the symptoms. We use cocaine self-administration (SA) model in rats and attempt to answer this question using a multi-disciplinary approach including *in vitro* brain slice electrophysiology, *in vivo* EEG recordings, sleep intervention, and behavioral tests of incubation of cocaine craving (time-dependent increases in cocaine seeking after withdrawal; Grimm et al., 2001). Following cocaine SA training (2 hr / d x 5 d) in young adult rats (P37-41 at start of training), the rats underwent withdrawal (WD) in the home cages while the EEG and EMG signals were chronically recorded. Compared to baselines (recorded prior to cocaine exposure), both NREM and REM total sleep times were reduced during WD day 1 - 3 weeks (wk), and trends of recovery were observed from wk 4 - 6. NREM and REM episode durations were both reduced in the light phase throughout the 6 wk WD. To enhance the consolidated sleep in the light phase, we used a custom-made treadmill system to sleep deprive (SD) the rats without significant exercise; and only during the dark phase. All animals were trained and tested during the dark phase, and allowed 3 days free of manipulations at the end of sleep interventions before any behavioral tests. We observed that 6-wk, or 3-wk (but not 1- or 2-wk) sleep interventions toward the end of the WD period significantly reduced cue-induced cocaine seeking without significantly affecting natural reward-associated learning (sucrose SA). E-phys recordings in *in vitro* brain slices revealed that the 3-wk sleep intervention significantly reduced the synaptic content of calcium-permeable AMPA receptors (CP-AMPARs) in nucleus accumbens medium spiny neurons, which has been shown at these synapses to critically regulate incubation of cocaine craving following long-term WD (Conrad et al., 2008; Loweth et al., 2014). By contrast, sleep fragmentation at 3 wk significantly enhanced the synaptic level of CP-AMPARs, as well as enhanced cue-induced cocaine seeking. Thus, sleep could bi-directionally regulate addiction-associated behaviors during abstinence with corresponding changes in the neurophysiology within the nucleus accumbens.

Disclosures: B. Chen: None. **Y.H. Huang:** None. **Y. Wang:** None. **Z. Liu:** None. **X. Liu:** None. **Y. Dong:** None.

Poster

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Support: NIH Grant DA021261

Title: ICV neuropeptide Y-induced decrease of cocaine-induced conditioned place preference in rats was not reversed by the Y-5 receptor antagonist CGP71683

Authors: *M. SUAREZ[1], K. BURKE[2], J. M. DIPIRRO[3], A. C. THOMPSON[4]
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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 results in neural adaptations that persist through the first few months of abstinence from chronic cocaine exposure. Our lab has recently found that, using immunocytochemistry, we have found a reduction of NPY-like immunoreactivity in areas of the brain that underlie cocaine-mediated behaviors. 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 the receptor specificity mechanism underlying the observed attenuation of cocaine-mediated behaviors in a model of cocaine exposure, an escalating dose regimen in rats. To this end, male Long-Evans (hooded) rats were given daily injections of cocaine (COC), using an escalating cocaine dose regimen (5-30 mg/kg IP for 21 days). CPP training occurred over the last 4 days of drug treatment and involved 2 conditioning sessions a day: one session with COC and one session with VEH. We used a two-chambered CPP apparatus in which each chamber had a different visual context. CPP testing was conducted 1, 7, and 21 days after the last cocaine treatment. ICV NPY microinjections were administered alone (0 or 0.3 nmol in 5µl) or in combination with NPY receptor antagonists (BIBO3304 [Y1], 10 nmol; BIIE0246 [Y2], 0.3 nmol; and CGP71683 [Y5], 15nmol) 30 min before the day 1 CPP test. We found that NPY alone blocked the expression of CPP and that co-administration with BIIE0246 or CGP71683 did not change the effect of NPY. Co-administration of NPY+BIBO further reduced the expression of preference. Co-administration of BIBO03304 and BIIE0246

was confounded by the appearance of motor impairments at higher doses. These results add to a growing body of literature which describes the regulation of cocaine-mediated behaviors by particular receptors of the NPY system.

Disclosures: **M. Suarez:** None. **K. Burke:** None. **J.M. DiPirro:** None. **A.C. Thompson:** None.

Poster

430. Cocaine Reinforcement II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 430.27/DD22

Topic: C.17. Drugs of Abuse and Addiction

Support: Ministry of Science and Technology of China 2014CB942801

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Title: Realtime manipulation of NAc GABAergic transmissions attenuates the expression of cocaine rewarding memory

Authors: *M. SHEN[1], L. WANG[2]
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Abstract: NAc D1+ and D2+ GABAergic medium-sized spiny neurons (MSNs) both form projections to palladium , while D1+ MSNs form direct projections to midbrain neurons as well. They are critical in rewarding and aversive learning, and that understanding the role of these NAc projections and the alteration of brain regions they targeted in the drug rewarding memory is of importance in the addiction research.In this study, we stimulated the GABAergic neurons in the NAc of mice expressing channelrhodopsin-2 under the control of the vesicular GABA transporter promoter precisely and temporally by an optogenetic approach, and examined its effects on the expression of cocaine-context-associated memory. *In vivo* opto-stimulation of the NAc GABAergic neurons in mice inhibited the expression of cocaine-induced CPP. On the next

day these mice were tested again and showed normal cocaine preference, confirming that the inhibition of NAc GABAergic on the cocaine CPP memory was transient and reversible. Stimulaing NAc GABAergic neurons inhibited the learning of cocaine-induced reinforcement as indicated by the behavioral sensitization. To explore how the cocaine rewarding memory was processed and integrated, we assessed the activity of NAc MSNs-targeted brain nuclei and found that the number of c-fos positive cells in ventral palladium was decreased when opto-activation of NAc GABAergic neurons during CPP expression, suggesting that the NAc GABAergic projections inhibit the ventral palladium activity and negatively regulate the expression of motivational effects induced by cocaine-context-associated cues.

Disclosures: **M. Shen:** None. **L. Wang:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.02/DD24

Topic: D.02. Auditory

Support: European Community Marie Curie fellowship (PIOF-GA-2011-300753)

Title: Role of the dorsal cochlear nucleus in spectral context effects: A computational approach

Authors: *B. FONTAINE
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Abstract: In perception, context is critical: a sensory input can be perceived differently, depending on the presence of other stimuli. A basic example is called enhancement. A harmonic complex (i.e. a series of tones at frequencies that are multiples of the same fundamental frequency) presented in isolation will be perceived as a single auditory object. If one tone is deleted from the complex (the conditioner) and then reintroduced (test), it will perceptually “pop out” as a separate sound object. This effect originates in the central nervous system and occurs preferably at a midrange of sound levels as in normal conversation. A manifestation of enhancement in speech perception is the negative after-image effect; if the sound preceding a

flat-spectrum harmonic complex has the inverted spectrum of a vowel (with troughs replacing peaks), this complex will be perceived as the vowel. Another phenomena that could be related to enhancement is the fact that a phoneme is understood differently depending on the spectral cues that precede it. We hypothesize that the dorsal cochlear nucleus (DCN) is the first stage where neural correlates of contextual effects emerge. While no clear evidence was found in the ventral cochlear nucleus, enhancement effects are not present one synapse before the DCN (in the auditory nerves (AN)) but are present one synapse after (in the inferior colliculus (IC)), making the DCN output neurons logical candidates. The DCN, called the cerebellum of the auditory system, has cortical features and its output cells, that project to the IC, receives lateral inhibition through a large number of interneurons. In this work, we implement a detailed model of a DCN population along the entire tonotopical axis receiving inputs from a state of the art AN model. We test whether the adaptation of the lateral inhibition in the DCN is sufficient to induce enhancement. We then test, using similar paradigm used in psycho-acoustical experiments on enhancement, negative after-image effect, and phoneme recognition, whether the DCN population responses can explain the perceived spectral context effects on auditory processing.

Disclosures: B. Fontaine: None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.03/DD25

Topic: D.02. Auditory

Support: Dutch Fund for Economic Structure Reinforcement (FES, 0908 'NeuroBasic PharmaPhenomics project')

Title: Chronic *in vivo* two-photon calcium imaging in the dorsal cortex of the mouse inferior colliculus

Authors: *J. G. G. BORST, H.-R. GEIS
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Abstract: Compared to the central nucleus, the function of the dorsal cortex of the inferior colliculus has been less well characterized. In mice, a large part of the dorsal cortex lies superficially, making it a favorable structure for chronic *in vivo* two-photon imaging. We

expressed the genetically encoded calcium indicator GCaMP6s (Chen et al, 2013) using adeno-associated viruses in C57BL/6J mice and repeatedly imaged sound responses of individual cells through a cranial window during periods exceeding one month. Responses to simple tones of different frequencies and intensities in awake, head-fixed mice showed that frequency response areas (FRAs) were often broad and complex, including areas in which the tones reduced intracellular calcium concentrations, probably as a result of inhibition of spontaneous firing. FRAs of individual cells within the same field of view typically differed considerably, suggesting sparse coding within the dorsal cortex. Repeated measurements of FRAs of the same neuron indicated that FRAs were generally stable. Behavioral work involving lesions in the cat dorsal cortex of the inferior colliculus has provided evidence for a role in auditory attention (Jane et al, 1965). We therefore tested whether the calcium responses to tones depended on behavioral state. Attentional level was monitored by measuring pupil sizes, electrocorticography and lick responses to tones presented in an operant conditioning task. In many cells, calcium responses to sound presentation were modulated by the behavioral state. The complex tuning properties and the modulation of firing properties by behavioral state suggests that the role of the dorsal cortex of the inferior colliculus in sound processing clearly differs from that of the central nucleus.

Disclosures: J.G.G. Borst: None. **H. Geis:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.04/DD26

Topic: D.02. Auditory

Support: NIH grant R01-DC000189 (DLO)

Title: Sound response properties of the optogenetically identified gabaergic neurons in the inferior colliculus (ic) of mouse

Authors: *M. ONO, D. C. BISHOP, D. L. OLIVER
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Abstract: The IC is a critical auditory center in the midbrain that contains functional zones with different sets of ascending inputs from the brainstem. These domains contain both glutamatergic and GABAergic neurons. The glutamatergic and GABAergic neurons are excitatory and

inhibitory respectively. Both neuron types project to the medial geniculate, so the consequences of their activity are totally different. To understand auditory processing in the IC, it is critical to know what information the glutamatergic and GABAergic neurons convey. However, it is unknown how these cell types respond to sound. To distinguish the GABAergic and glutamatergic neurons *in vivo*, we used VGAT-channelrhodopsin (VGAT-ChR2) mice with light stimulation in single unit recordings. In the IC of VAT-ChR2 mice, our immunohistochemical analysis showed that GABAergic neurons exclusively expressed the ChR2, and the glutamatergic neurons did not. IC neurons were selected at random and were exposed to blue light stimulation (473 μm, 100-150 ms, 10-50 mW). IC neurons had one of two responses to light: neuronal firing was either excited by the light stimulus or suppressed by the light and were assumed to be GABAergic or glutamatergic, respectively. To study how GABAergic and glutamatergic neurons differ in their responses to sound, we examined their frequency tuning, firing rates, binaural responses, and responses to amplitude modulated (AM) sound. The results showed that the light-identified GABAergic neurons were heterogeneous in their responses to sound, as were the light-identified glutamatergic neurons. The GABAergic and glutamatergic neurons differed in their temporal response properties, but did not show a significant difference in frequency tuning, firing rate, and binaural responses. Most GABAergic neurons preferred modulation frequencies below 64 Hz and rarely respond to higher modulations. On the other hand, glutamatergic neurons often respond to modulation frequencies >64 Hz and some neurons showed high pass properties that was not found in GABAergic neurons. The presence of an off response in some glutamatergic neurons but not in GABAergic neurons was another difference between cell types. These results suggested that inhibitory tectothalamic pathway from the IC conveys information similar to the excitatory pathway in many cases. Exceptions may be the absence of tectothalamic inhibition for high modulation frequencies and off responses.

Disclosures: **M. Ono:** None. **D.C. Bishop:** None. **D.L. Oliver:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.05/DD27

Topic: D.02. Auditory

Support: NIH-NIA Grant P01 AG009524

Title: Long-term treatment with progestin does not affect auditory midbrain receptive fields in old CBA mice

Authors: *J. M. MANSOUR[1], B. H. GROSS[2], E. J. BRECHT[1], X. ZHU[1], T. T. WILLIAMSON[1], R. D. FRISINA[1,2], J. P. WALTON[3,2]
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Abstract: The present study focuses on how hormone replacement therapy (HRT) affects auditory coding, particularly: Are the negative effects on hearing and central auditory processing reversible after HRT treatments are terminated? We hypothesized that aging female mice receiving progesterone will improve their hearing upon the cessation of treatment, reflecting a release from the deleterious effects of progesterone. As these hormones are known to modulate neurotransmitter activity, particularly GABAergic inhibition, it was hypothesized that alterations in receptive fields at the level of the inferior colliculus (IC) would be evident. Four middle-aged female ovariectomized CBA mice received subcutaneous placement of two consecutive 90-day time released pellets of progestin (0.4 mg/day). Control data included two females implanted with placebo pellets, and three age-matched males with no treatment. Mice were allowed to recover for two months at which time they were 23-24 months of age. Single- and multi-unit extracellular physiological activity was recorded using a vertically oriented 16-channel silicon probe. The electrode was positioned stereotaxically over the IC, and advanced dorsoventrally into the central part of the IC. Excitatory frequency response areas (eFRAs) were acquired using tone burst stimuli, 25-msec in duration with 5-msec rise/fall times. Each of the 2125 frequency by intensity combinations (125 frequencies from 2 to 64 kHz in 500 Hz steps; 17 intensities from 0 to 80 dB SPL in 5 dB steps) were presented 5 times in a random order at a rate of 10/sec. We quantified eFRAs using the best frequencies (BF), minimum threshold (MT), and measured tuning width using Q10 & Q40. We found no significant differences for mean BFs or MTs for all three subject groups. Specifically, mean BFs were 19 kHz, 20 kHz, 24 kHz and MTs were 38 dB, 34 dB, 41 dB for progestin, placebo, and male, respectively. The tuning data likewise were similar across all three groups where the average width of the receptive fields for Q10 were 14.6kHz, 14.6kHz, 15.2kHz and Q40s were 28.2kHz, 27.7kHz, 22.6kHz for the progestin, placebo, and male, respectively. These results suggest that progestin did not have detrimental effects on IC following HRT and a 2 month post-recovery period. Considering previous findings as well, this suggests that progestin may affect hearing negatively only in combination with estrogen, but not independently. Ongoing studies are aimed at comparing these findings for two additional subject groups, estrogen + progesterone, and estrogen only, to more fully understand the effects of HRT, and hormone levels in general, on sensory processing.

Disclosures: J.M. Mansour: None. **B.H. Gross:** None. **E.J. Brecht:** None. **X. Zhu:** None. **T.T. Williamson:** None. **R.D. Frisina:** None. **J.P. Walton:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.06/DD28

Topic: D.02. Auditory

Support: National Basic Research Program (973) of China 2011CB933204

Title: Neural responses to spectrotemporal patterns in auditory midbrain: from simple to complex

Authors: W. TANG, R. XU, *B. HONG
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Abstract: Neurons in primary visual cortex (V1) are commonly classified as simple and complex cells based on their selective and invariant responses to visual stimuli. Auditory neurons are known to be sensitive to spectrotemporal (ST) patterns of acoustic stimuli, but where the invariant responses to ST patterns emerge in the ascending auditory pathway is unclear. Using single-unit recordings from the central nucleus of inferior colliculus (CNIC) of the anesthetized rats, we found that response profiles of neurons in CNIC showed a similar distinction as V1 neurons: simple neurons (65%, 93/143) in CNIC were selective to ST patterns, whereas the responses of complex-like neurons (35%, 50/143) showed some invariant properties. Employing the framework of linear-nonlinear (LN) models, we further characterized the ST receptive fields (STRFs) of both classes of neurons. The response of a subset (25.8%, 24/93) of simple neurons was best characterized by 1D LN models, whereas other simple neurons (74.2%, 69/93) were best described by 2D LN models with additional components, which increased the selectivity to ST patterns. On the other hand, the invariant properties of complex-like neurons were captured by multiple filters. We compared the LN models to spike-triggered averaged (STA) model, the traditional method for STRF analysis, in terms of prediction accuracy. We found that the LN models predicted novel responses significantly better than the STA model, which suggested that the LN models provided a more accurate description of the response properties of CNIC neurons. Finally, the response properties and receptive fields of all units were compared between classes. We found that complex-like neurons had significantly lower averaged firing rates ($P < 0.0001$, Mann-Whitney U-test), broader frequency bandwidths ($P < 0.005$) and longer latencies ($P < 0.005$). These results suggest that a hierarchy of increasing selectivity and invariance of neural responses to ST patterns emerges in auditory midbrain.

Disclosures: **W. Tang:** None. **B. Hong:** None. **R. Xu:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.07/DD29

Topic: D.02. Auditory

Support: NIH DC011580

American Federation for Aging Research

Title: Age-related changes in the transformation of responses to amplitude modulated sounds in the inferior colliculus

Authors: A. PARTHASARATHY, *E. L. BARTLETT
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Abstract: Age-related changes in auditory temporal processing typically involve elevated hearing thresholds and reduced afferent drive due to cochlear damage and synaptic deficits. Less well understood are the changes in neural processing in the central auditory pathway that are thought to be due to compensatory increases in neuronal excitability. In this study, putative central changes in processing sinusoidally amplitude modulated noise (nAM) are investigated through a combination of three measures in a rodent aging model. Envelope following responses (EFRs) to nAM stimuli changing in amplitude modulation frequency and depth provide an assessment of the overall changes seen at a population level. Local field potentials (LFPs) from the inferior colliculus (IC) of these same animals to the nAM stimuli provide an estimate of the local circuit tuning, including the presynaptic inputs, coming in to the neurons. The single unit spiking outputs from the IC neurons are recorded concurrently. Age-related deficits in synchronous representations of the AM stimuli were observed in all three metrics, typically for faster modulation frequencies. Comparing the LFPs to the corresponding unit responses revealed an age-related change in the transformation of the responses in the IC. The aged neurons showed a strong correlation between the LFPs and the single units, suggesting minimal processing between the IC inputs and outputs. This correlation was absent in the young for many modulation frequencies. These results imply that the IC in young animals shapes the incoming

responses to become more selective, potentially using synaptic depression or inhibitory mechanisms, while this transformation becomes degraded with age.

Disclosures: A. Parthasarathy: None. **E.L. Bartlett:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.08/DD30

Topic: D.02. Auditory

Support: Advance Seed Grant

NSF

Title: Differing effects of noise on subcortical speech representation in younger and older adults

Authors: K. ERHARDT, A. PRESACCO, J. SIMON, *S. ANDERSON
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Abstract: Younger adults experience little difficulty comprehending speech when attempting to engage in conversation in a noisy environment. Conversely, many older adults report having trouble understanding what is being said, even when speech is audible. Loss of neural synchrony with age may lead to deficits in auditory temporal processing. Electroencephalography can be used to analyze the effects of noise on subcortical processing. We hypothesized that frequency following response (FFR) amplitudes are noticeably degraded for speech stimuli presented in noise vs. quiet in younger adults; however, this noise-induced degradation is not seen in older adults because pre-existing temporal processing deficits desynchronize their responses in both conditions. Participants comprised 15 young adults (21 - 30 years old) and 15 older adults (60 - 74 years old) with normal hearing. FFRs were recorded in response to a 170 ms speech syllable (/da/) presented diotically, with a fundamental frequency of 100 Hz at 80 dB SPL in two separate conditions: quiet and noise. The noise condition added a single talker narrating a story. Participants' sentence recognition in noise was behaviorally assessed using the Quick Speech-in-Noise test (QuickSIN). Significant differences in the FFR were noted between younger and older adults. Older adults have lower RMS and spectral amplitudes than younger adults, but greater effects of noise are found in the younger adults. Specifically, the presence of noise reduced

overall amplitudes of the temporal envelope in the time and frequency domains mainly in younger adults. Temporal fine structure was also significantly reduced by noise, but only in younger adults. Younger adults had significantly lower thresholds on the QuickSIN than older adults. The lack of quiet-to-noise differences in the FFR of older adults suggests that they may be affected by central auditory processing deficits. In crowded environments, competing stimuli are suppressed to attend to the target speech signal; therefore, older adults' temporal deficits and lack of suppression in noise reduce their ability to focus on the target speaker. These observations are consistent with the results from the QuickSIN test, which revealed poorer scores for older adults. Altogether, our results suggest a decline in temporal precision that may partially account for older adults' difficulty understanding speech in noise.

Disclosures: **K. Erhardt:** None. **S. Anderson:** None. **A. Presacco:** None. **J. Simon:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

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Program#/Poster#: 431.09/DD31

Topic: D.02. Auditory

Support: NIH DC00436

NIH P30 DC0466

NIH DC00046

Title: Tonotopic organization of the turtle brain stem

Authors: *K. L. WILLIS[1], A. JOHNY[2], C. E. CARR[2]
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Abstract: In mammals, birds, and alligators, the auditory nuclei are organized tonotopically. This may not be the case in fish, which begs the question of when in vertebrate history tonotopic organization first appeared. Turtles are relevant to this because current phylogenetic analyses suggest turtles are a sister group to the archosaurs, and further suggests an aquatic origin for this group. and have similar ascending auditory pathways to birds and crocodiles. Tonotopy is possible, because hHair cells in the turtle inner ear are tonotopically organized. In order to see if their tonotopic arrangement was preserved in the brain stem, we examined tonotopy both

anatomically and physiologically. We hypothesized that caudal nucleus magnocellularis (NM) will process low frequency sounds and rostral NM will process higher frequency sounds, as found in archosaurs. An isolated head preparation was used to characterize tonotopy physiologically. Calibrated earphones were sealed around the tympanum for sound delivery. The preparation was maintained using artificial cerebrospinal fluid (ACSF) and remained stable throughout the experiment (up to 12 hours). Single unit recordings were made from NM. To anatomically characterize tonotopic projections, we used NeuroVue, Neurobiotin (NB), and fluorescent dextran injections into opposite ends of the basilar papilla and auditory nerve. Auditory responses from NM neurons revealed best frequency selectivity from 100-450 Hz. Anatomically, NM received input from the auditory nerve, in the form of en passant or bouton terminals (areas of 5.4 ± 0.78um2), located on both cell bodies and neuropil. Reconstructions from NB injections into the auditory branch of VIII nerve revealed terminals in NM that were not significantly larger (5.437 ± 2.3 μm2) than those on NA (5.326 ± 2.8μm2, p=0.34; NA n = 135, NM n = 220). Terminals onto NA were, however, significantly more round (form factor = 0.84± 0.07) than those on NM (form factor = 0.78±0.13, $p < 0.001$), as determined by ANOVA. No end bulbs were found, consistent with observations in the low frequency portions of avian NM. If turtles reveal a tonotopic distribution of neurons in NM and NA that is similar to that of archosaurs, then this will support the evolutionary relationship between turtles and archosaurs and also provide insight into how the evolution of tonotopy.

Disclosures: K.L. Willis: None. **A. Johny:** None. **C.E. Carr:** None.

Poster

431. Auditory Processing: Temporal, Frequency, and Spectral Processing- Model Systems and Subcortical

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 431.10/DD32

Topic: D.02. Auditory

Support: NSF

CRCNS

Title: Sensori-motor neural activity in the Superior Colliculus of freely-flying bats

Authors: N. B. KOTHARI[1], M. J. WOHLGEMUTH[2], *C. F. MOSS[3]
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Abstract: The superior colliculus (SC) is a laminated midbrain structure implicated in species-specific orienting behaviors. The superficial layers of the SC receive retinal input, intermediate layers show multisensory and sensorimotor properties, and deep layers exhibit pre-motor activity. Previous research on the bat SC has revealed specialized properties linked to spatial orientation by sonar: Auditory neurons in bat SC show selectivity to stimulus azimuth, elevation and echo delay, and microstimulation elicits sonar vocalizations. In addition, premotor neurons fire before each sonar cry. These findings were obtained from stationary bats, and here we extend studies of the bat SC to free-flying animals. We trained big brown bats to locate a platform and land on it. Single unit activity was recorded across the SC laminae, while high-speed audio and video recordings captured the bat's echolocation and flight behaviors. Neural activity was then correlated with the sonar vocalizations, as well 3D head aim and position in space. We find that the superficial layers show neural activity correlated with echo input, while the intermediate and deeper layers show sensorimotor and pre-motor activity, respectively. Integration of information across each of these functional layers would be essential for enabling behaviors required for spatial navigation, orientation and prey capture. Additionally, in order to reconstruct the timing of the echoes arriving at the bat's ears, we developed an echo model using the recorded sonar vocalizations, the bat's 3D head aim and position in space. Correlating the echo timing information with neural activity we can characterize neurons which respond to echo arrival times of different objects in the bats flight trajectory.

Disclosures: N.B. Kothari: None. **C.F. Moss:** None. **M.J. Wohlgemuth:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 432.01/EE1

Topic: D.03. Multisensory

Title: Dissecting fly proboscis circuitry to explore the sensorimotor coordination of a flexible motor program

Authors: *C. E. MCKELLAR, J. CANNON, J. H. SIMPSON
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Abstract: Complicated behavioral programs can be divided up into smaller motor modules, which may be recruited by a variety of behaviors. However, the organizing principles of how neural circuits driving different behaviors can call a common motor output are largely unexplored. Here, we use a model system of *Drosophila* proboscis extension, a motor action employed during many behaviors, such as feeding, drinking, courtship, and grooming. We have found that proboscis extension is not a simple reflex but is composed of modules that vary between different behavioral contexts. It employs flexible coordination, and has the potential to serve as a model for directed reaching movements in an articulated limb. We have developed a library of fly strains that target specific proboscis motor neurons, allowing precise manipulation of motor subroutines to understand this coordination. With such an invertebrate model of directed reach, sensorimotor integration can be explored not just at the level of a single synapse, but at the level of circuit architecture. To understand this circuit architecture, we have conducted a screen to identify new components of proboscis control circuits within the brain. By activating pools of neuron types, we triggered a rich spectrum of proboscis motor behaviors. We have found several new interneurons that participate in facets of this behavior, and are using both anatomical and behavioral strategies for putting them together into their circuit contexts. This approach of identifying many of the neuron types involved in a behavior in a high-throughput manner may ultimately reveal how upstream neurons converge on common motor elements, or potentially discover command circuitry that triggers whole behavioral routines.

Disclosures: **C.E. McKellar:** None. **J.H. Simpson:** None. **J. Cannon:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

Location: Halls A-C

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Program#/Poster#: 432.02/EE2

Topic: D.03. Multisensory

Support: JSPS KAKENHI Grant 12J10557

Title: Analysis of locomotion-related neural activity in odor source searching behavior of an insect

Authors: *R. MINEGISHI[1], D. KURABAYASHI[1], R. KANZAKI[2]
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Abstract: Animals can execute task behavior even though they don't have any previous knowledge about environment. To understand the neural processing in a brain underlying such animal behavior is one of the fundamental goals in neuroscience. We focused on the odor source searching behavior of male silkworm moth, *Bombyx mori*. Searching behavior of silkworm moths is only triggered by their conspecific female pheromone sensed by their antennae. Unlike other mots, they can't fly. Their behavior consists of sequences of surge and turn in locomotion. These features are merits to analyze its behavior. In addition to the behavioral analysis merits, their olfactory neural circuits in a brain underlying this searching behavior are well studied from sensory input region to premotor region. So far, phasic and long-lasting excitation/inhibition patterns of single neurons in protocerebral region were presumed to relate to locomotion. However, direct relationships between neural processing in the brain and behavior have not yet been examined by recording them at the same time. In this study, we performed simultaneous measurement of neural activities and locomotion behavior of moths by implanting wire electrodes into the brain. We tethered the moths on a Styrofoam ball floated by air, and locomotion data were acquired by optical mouse sensors adjacent to the ball. We triggered behavior of moths by olfactory stimuli. We also examined the effects of other modal stimuli, i.e. visual stimuli on olfactory triggered neural activities. We gave moths optical flow stimuli as visual information. We recorded neural signals from premotor region, lateral accessory lobe in the protocerebrum of a brain. We checked the recording sites stained by 4% Lucifer yellow applied to the tips of the electrodes. Neural activities acquired by extracellular recording were sorted into unit activities. We classified the units into which relating to turn velocity and forward velocity during walking. We analyzed the time delay between the change of firing rate of the units and the change of walking velocities based on their correlation. We also analyzed and evaluated the effects of visual stimuli on the change of the firing rate of the units relating to turn velocity and forward velocity in locomotion.

Disclosures: **R. Minegishi:** None. **D. Kurabayashi:** None. **R. Kanzaki:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

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Topic: D.03. Multisensory

Support: We thank Eva Naumann, Florian Engert and Adam Douglass for providing the aequorin-GFP transgenic lines and helpful comments

Title: Closed-loop sensorimotor integration in the moving spinal cord

Authors: *S. KNAFO[1,2,3], O. THOUVENIN[4], H. PASCAL-MOUSSELARD[5], C. WYART[4,3,2]
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Abstract: Sensorimotor behaviors are by definition closed-loop processes, during which sensory feedback modulates ongoing locomotor activity. Traditional "fictive preparations", in which the spinal cord is isolated and/or the animal paralyzed, only provides an "open-loop" access to spinal circuitry. We developed a bioluminescent approach for monitoring neural activity during ongoing active locomotion. We monitored the global neural activity of genetically targeted populations of spinal neurons using the bioluminescent reporter aequorin-GFP while simultaneously recording auditory-vestibular (AV) evoked locomotor activity using a high-speed (1000 Hz) infrared camera in 3 and 4 days post-fertilization zebrafish larvae. Automatic analysis of bioluminescence signals and classification of locomotor activity were performed. We compared the recruitment of spinal motor and sensory neurons, as evidenced by their bioluminescent signals, while the animal achieved a closed-loop "active" versus an open-loop "fictive" sensorimotor behavior in actively moving and paralyzed animals. Recruitment of spinal motoneurons during active AV-evoked locomotion correlated with automatic movement classification: escape only (ER), slow swim only (SS), escape followed by a slow swim (ER+SS). We observed a steeper bioluminescence rising slope and shorter bioluminescence decay for ER compared to SS. In open-loop fictive behaviors, recruitment of spinal motoneurons was slower and the decay constant of their bioluminescence signal was longer compared to closed-loop active behaviors. We are currently investigating the activity of spinal sensory neurons during active locomotion. Preliminary results show that Rohon-Beard spinal somatosensory neurons are recruited during active behaviors but not during fictive assays, as evidenced by the suppression of their bioluminescence signal. These results show a differential recruitment of motor and sensory spinal neurons during active compared to fictive locomotion using genetically targeted reporters. They emphasize the importance of studying spinal sensorimotor integration in actively moving animals in order to apprehend closed-loop neural processes.

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Poster

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Topic: D.03. Multisensory

Title: Behavioral and neuronal studies of navigation tasks in zerbafish larva

Authors: *R. OLIVE, R. CANDELIER, G. DEBRÉGEAS
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Abstract: Zebrafish larvae at 5-8 days post-fecundation exhibit a large panel of innate behaviors including rheotaxis, counter-current swimming and predator escape. To discriminate among different situations and adopt an appropriate response, the animal gathers and combines inputs from two different sensory systems: the visual system - through, e.g. the optomotor reflex - and the lateral line, the mechanosensory organ that mediates flow perception. To investigate the neural processes at play during rheotaxis and counter-current swimming, we have adopted two complementary experimental approaches in which we recorded responses to continuous, radial flows generated by a suction point. First, in shallow end behavioral assays we tracked a large number of freely-swimming larvae (N>3000) in different conditions where we tuned visual perception (light/dark) and flow perception (neuromast ablation). Experiments without neither flow nor visual cues showed no response, suggesting that the vestibular system is not exploited. The analysis of high-frequency body curvature traces revealed how series of short swim bouts are modulated (in terms of number of bouts and frequency inter- and intra-series) in the different conditions. Although visual cues are sufficient to perform efficient counter-current swimming, we established that the animal also uses information coming from the lateral line to refine its swimming patterns. Then, we have developed a custom tethering setup in which the tail of the animal is freed and monitored neural activity with a Single-Plane illumination Microscope (SPIM) during flow perception tasks. This SPIM setup allowed us to record with single-cell resolution the dynamics of large neuronal assemblies (N>20000) in transgenic zebrafish larva expressing a genetically encoded calcium indicator (GCaMP). Neuronal activity has been investigated along the whole perceptive pathway in response simple suction stimuli, and analyzed statistically across several trials and individuals. This opens the way for a precise description of neuronal integration of lateral line information in experiments in which more complex flow patterns are generated and/or visual cues are delivered simultaneously.

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Poster

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Title: Auditory cortex controls innate defense behavior via corticofugal circuits

Authors: *X. R. XIONG, F. LIANG, X. JI, B. ZINGG, L. A. IBRAHIM, H. W. TAO, L. I. ZHANG
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Abstract: Defense against environmental threats is essential for animal survival. While previous studies have been largely focused on the neural circuitry for learned defensive behaviors such as in fear conditioning, innate defense circuits responsible for the transformation of unconditioned sensory stimuli and the role of sensory cortices in generating defensive behaviors remain elusive. Here, we show in mice that auditory cortex drives an innate sound-induced fleeing behavior via corticofugal projections to the inferior colliculus (IC). Optogenetic activation of corticofugal axon terminals in the IC is sufficient to initiate the defensive behavior, while optogenetic inhibition of these axons reduces the sound-triggered behavioral response. The corticocollicular axons monosynaptically innervate neurons in the IC cortex, which directly drive structures of the midbrain defense system, including the dorsal periaqueductal gray (PAGd). Suppression of activity of IC cortex neurons reduced the sound-triggered fleeing response, while activation of IC cortex neurons or axonal terminals from the IC cortex in the PAGd is sufficient for provoking the fleeing behavior. Together, our study reveals an innate defense circuit in which the corticocollicular projection can transmit threatening sensory stimuli for the auditory cortex to drive innate acoustic-motor responses. Given the prevalence of corticofugal projections, this previously unrecognized role of corticofugal circuits may be general across sensory modalities.

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Poster

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Title: Cross-modal sensory interactions in the thalamic reticular nucleus: a neural basis for cross-modal modulation of attention and perception

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Abstract: The thalamic reticular nucleus (TRN), a cluster of GABAergic cells innervated by collaterals of thalamocortical and corticothalamic projections, occupies a highly strategic position to exert gain and/or gating control of sensory processing in the thalamocortical loop. Given that cross-modal sensory interaction takes place in the TRN, the TRN could play a pivotal role in cross-modal modulation of attention and perception. In fact, diverse subthreshold cross-modal interactions between visual and auditory inputs take place in the TRN (Kimura, 2014). To determine whether this cross-modal sensitivity of the TRN could extend across sensory modalities, the present study examined interactions between auditory and somatosensory inputs in single TRN cells using juxta-cellular recording and labeling techniques. Experiments were performed on anesthetized rats. Auditory (noise burst, 45-80 dB, 100 ms) or somatosensory (electrical stimulation of the hind paw, 1-3 mA, 1 ms) stimulation alone and combined stimulation with a temporal gap (0-600 ms) were randomly given. Sensory response and spontaneous cell activity were recorded at random. Cells were labeled with biocytin or neurobiotin. Recordings were obtained from 132 cells that included 86 auditory cells responsive only to sound, 15 somatosensory cells responsive only to electrical stimulation, 22 bi-modal cells and 9 cells responsive only to combined stimulation. Auditory or somatosensory response (unit discharges) was modulated by electrical stimulation or sound, which did not elicit unit discharges, i.e., subthreshold somatosensory or auditory input, in the majority of cases (80 auditory and 13 somatosensory cells). Suppression predominated in modulation that took place not only in primary responses but also in late responses repeatedly evoked after sensory stimulation. Combined stimulation also evoked de-novo responses, and modulated response latency and burst spiking. The phase of oscillatory response was also shifted by combined stimulation. Cells showing modulation of the sensory response were distributed in the whole auditory and the caudal somatosensory sectors of the TRN. Bi-modal cells were mostly found in the somatosensory sector. Modulated cells sent axonal projections to first- or higher-order

thalamic nuclei that compose the lemniscal and non-lemniscal sensory systems. The results suggest that the TRN incorporates sensory inputs of different modalities into single cell activity across sensory modalities. The TRN is thought to exert potentially very extensive cross-modal control of sensory processing that contributes to cross-modal modulation of attention and perception.

Disclosures: A. Kimura: None. **H. Imbe:** None.

Poster

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NIH DC007703

Title: Taste cortex influences olfactory processing

Authors: *J. X. MAIER, D. B. KATZ
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Abstract: Sensory systems are typically studied in isolation, yet rarely act alone. In line with previous work on multisensory integration in the visual and auditory systems, our work has shown that taste-smell interactions involve functional connections at the level of primary sensory cortex. Here we demonstrate that such intrinsic multimodal connectivity is not only responsible for sharing modality-specific information across sensory systems but also affects unisensory processing. First, we recorded spiking activity simultaneously from neurons in primary olfactory (OC) and gustatory (GC) cortex of awake rats in response to taste stimuli. Pairs of taste-selective neurons across OC and GC exhibited trial-by-trial correlations in firing rate, and taste-selective activity in GC preceded taste-selective activity in OC, suggesting that taste-related input to OC arrives via GC. When we then suppressed neural activity in GC (GCx) in a subset of trials (through optogenetic stimulation of the inhibitory channel ArchT), taste-selectivity of OC neurons was significantly decreased, confirming that GC provides a major source of taste-related input to OC. To further test the implications of functional connectivity with GC for neural processing in OC, we recorded spontaneous and odor-evoked neural activity in the same

paradigm. During GCx trials, both spontaneous and odor-evoked activity was increased in as many OC neurons (and to a similar degree) as it was decreased, suggesting that GCx modulates (rather than impairing) neural processing in OC. These findings demonstrate that GC not only provides taste-related input to OC, but plays an integral role in olfactory processing, even in the absence of taste stimuli. Ongoing work will determine how such intrinsic multimodal connectivity affects odor perception.

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Poster

432. Multisensory: Neural Circuitry and Connections

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Title: Unisensory inputs during sensitive periods control the multisensory development via directed interactions within cortico-cortical networks

Authors: *I. L. HANGANU-OPATZ[1], B. RÖDER[2], K. SIEBEN[1]
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Abstract: Optimal behavior of humans and animals requires the integration of inputs across senses. Cross-modal integration is mediated by both thalamo-cortical and cortico-cortical interactions. In contrast to unisensory development, the mechanisms underlying the ontogeny of cross-modal interplay and its dependence on experience are still unknown. Here, we elucidate the critical factors controlling the development of visual-somatosensory interactions and their behavioral correlates by performing extracellular recordings of network activity in primary sensory cortices *in vivo*, identifying the anatomical substrate, assessing the coupling strength and directionality of cortico-cortical communication, and testing the cross-modal novelty recognition in pigmented rats. Similar to adult animals, juvenile rats without cross-modal experience displayed a supra-additive augmentation of evoked responses and phase reset of network oscillations in response to cross-modal light and whisker stimulation. In contrast, adult rats with

a history of neonatal tactile (i.e. whisker) deprivation showed diminished cross-modal evoked responses. Moreover, these neonatally deprived rats demonstrated a decreased coupling by synchrony and altered directed interactions between primary sensory cortices that resulted from sparsification of direct visual-somatosensory projections. At the behavioral level, these structural and functional deficits led to an impairment of cross-modal object recognition. Thus, unimodal experience during early development is necessary for setting up the thalamo-cortical and cortico-cortical networks accounting for multisensory processing. We suggest that these mechanisms represent the substrate of multisensory impairment in humans with a history of early unimodal deprivation.

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Poster

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Wellcome Trust

Action on Hearing Loss

Title: Multisensory integration in ferret auditory cortex: Effects of inactivating visual cortex

Authors: *S. M. TOWN, K. C. WOOD, G. P. JONES, H. ATILGAN, J. K. BIZLEY
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Abstract: A proportion of neurons in ferret auditory cortex respond to simple visual stimuli, and auditory responses to broadband noise can be modulated by the presence of a visual stimulus (Bizley et al., 2007). Visual interactions in auditory cortex may originate from many areas: anatomically inputs originate from visual cortex, parietal cortex and the suprageniculate nucleus of the thalamus (SGN). However, the functional relevance of these inputs remains to be determined. Here, we investigated the role of a sub-region of visual cortex - the suprasylvian cortex (SSY) - which projects strongly to the auditory cortex in particularly to the non-primary auditory fields located on the anterior bank of the Ectosylvian Gyrus. Multi-unit neural activity was recorded from auditory cortex of three ketamine-medetomidine anesthetized ferrets before,

during and after inactivation of SSY using cooling loops that reduced the cortical surface temperature to between 4 and 7C. Simultaneous recording of neural activity within SSY of two ferrets confirmed the efficacy of inactivation through cooling. Auditory cortical units were found in which SSY cooling significantly reduced visual but not auditory responses. Furthermore units were found in which visual modulation of auditory responses was selectively abolished by cooling. We also found evidence that regions other than SSY drive visual activity in auditory cortex as several units demonstrated visual responses robust to cooling. Examples were also discovered in which cooling reduced visual responses but only within a circumscribed time period of the response. A small number of auditory cortical units showed cooling induced visual responses or visual modulation of auditory responses. These findings support the role of SSY in multisensory integration within ferret auditory cortex, but also indicate the involvement of additional brain regions and a broader network of regions of which SSY is part. To determine the role of direct projections from SSY to auditory cortex (as opposed to projections that affect auditory cortex through intermediary brain regions), ongoing optogenetic experiments using virally-transduced expression of ArchT are focussed on selectively inactivating SSY neurons sending axons to auditory cortex. Using the same viral route to express GFP, we are also mapping the projections of SSY neurons in order to determine candidate brain regions that may contribute to a larger scale multisensory network that controls visual activity in auditory cortex.

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Poster

432. Multisensory: Neural Circuitry and Connections

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Title: Development of a rat model for studying cortical multisensory processing

Authors: A. SCHORMANS, M. TYPLT, *B. L. ALLMAN
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Abstract: Throughout the mammalian cortex there are functionally-specialized regions that are largely populated by neurons capable of integrating information from more than one sensory

modality. Importantly, previous studies on carnivores and non-human primates have revealed that that not all neurons in a given multisensory cortical area are equally responsive to multisensory stimuli. For example, within areas of the cat extrastriate visual cortex, there are (1) "bimodal" neurons which show suprathreshold spiking responses to both auditory and visual stimuli, (2) "subthreshold multisensory" neurons which do not respond overtly to auditory stimulation, but show an enhanced response to a visual stimulus when it is paired with an auditory cue, and finally (3) "unisensory" neurons which are only affected by visual stimuli. Because we are proposing to use a rat model to further uncover the anatomical and physiological mechanisms responsible for this variable multisensory responsiveness, our first efforts have been to profile the response characteristics of single neurons in the rat lateral extrastriate visual cortex (V2L) as well as the neighboring dorsal auditory area (AuD). In the present study, adult male Sprague Dawley rats were anesthetized with ketamine/xylazine, and extracellular electrophysiological recordings were performed during a quantitative multisensory testing protocol consisting of auditory and visual stimuli, presented alone and in combination to determine the spiking response profile for each neuron (i.e., whether the neuron would be classified as unisensory, subthreshold or bimodal). Fairly consistent with previous results in other species, approximately 50% of the neurons recorded in the rat V2L and AuD could be classified as multisensory, with more neurons showing bimodal response characteristics than subthreshold multisensory effects. Furthermore, only a limited proportion of these bimodal neurons showed "multisensory integration" in which their response to the combined audiovisual stimuli was significantly different than that observed for the most effective single modality stimulus. Collectively, our findings confirm that the rat represents a viable model for future studies investigating the anatomical/physiological mechanisms of cortical multisensory processing in mammals.

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Poster

432. Multisensory: Neural Circuitry and Connections

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Title: Cross modal modulation of orientation selectivity in mouse visual cortex

Authors: *L. IBRAHIM[1,2], X. JI[1], L. MESIK[1,2], Y.-T. LI[1,2], L. I. ZHANG[1,2], H. W. TAO[1,2]
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Abstract: Different cortical regions are interconnected with long-range intracortical axons. How sensory processing is modulated by inputs from other cortical areas, esp. those of different modalities remains not well understood. Using viral tracing methods, we observed fibers originating in layer 5 of the primary auditory cortex (A1) and projecting to the superficial layers of the primary visual cortex (V1). We expressed floxed AAV2-Channelrhodopsin2 (ChR2) in the A1 of a layer 5-specific Cre mouse line and explored how optogenetic activation of this projection pathway influences visual processing in different layers of the V1. Using *in vivo* loose-patch recording as well as Ca2+ imaging, we found significant sharpening of orientation tuning of excitatory neurons in layer 2/3, but not in layer 4. This phenomenon was more robust under low contrast than high contrast stimuli, and it was also observed when the mouse was presented with visual stimulation coupled with sound stimulation. In acute brain slices, optogenetic activation of A1 fibers in the V1 revealed direct excitatory synaptic inputs to layer 1 inhibitory neurons as well as layer 2/3 excitatory neurons. *In vivo*, we confirmed that both sound stimulation and optogenetic activation of A1 fibers caused a robust increase of firing rates of layer 1 neurons. Since layer 1 neurons are known to inhibit layer 2/3 neurons, we postulate that the excitatory neurons are generally inhibited by layer 1 inputs activated by A1 fibers. We are currently investigating how changes in the balance between excitation and inhibition by A1 inputs may lead to sharpening of the orientation tuning of layer 2/3 excitatory neurons.

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Poster

432. Multisensory: Neural Circuitry and Connections

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Title: Reversible deactivation of motor cortex reveals functional connectivity with posterior parietal cortex in the prosimian galago (Otolemur garnetti)

Authors: *D. F. COOKE[1,2], I. STEPNIEWSKA[3], D. J. MILLER[3], J. H. KAAS[3], L. KRUBITZER[1,2]
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Abstract: A complex network of connections between motor (M1), premotor (PM) and posterior parietal cortex (PPC) in primates plays an important role in voluntary movement. Long-train intracortical stimulation (ICMS) in all of these fields elicits complex movements, which are segregated into domains of ethologically relevant behaviors such as grasping, reaching or defense. Additionally, M1, PM and portions of PPC send direct projections to the spinal cord in primates. Recently, chemical deactivation of M1 and PM in New World monkeys and prosimian galagos has been shown to alter the movements evoked by stimulation of PPC, suggesting that inputs from M1-PM strongly modulate neurons in PPC. While these studies are intriguing, it is difficult to determine the extent of chemical deactivation, which can be quite large, and it takes hours to reverse the effects of this type of deactivation. The goal of the present investigation was to rapidly and reversibly deactivate forelimb (e.g. reach, grasp, lift) and orofacial domains (e.g. grimace) of M1 by cooling, and examine the effect of such deactivation on movements evoked from PPC in 4 anesthetized galagos. By using very small (1x2 mm or 2x3 mm) microfluidic thermal regulators we could control both the spatial extent and duration of cooling deactivation and make minute-by-minute observations of the resulting effect. Similar to previous studies, we demonstrate that deactivation of an M1 forelimb domain (e.g. forelimb lift/reach) decreases the amplitude of or eliminates movement evoked by ICMS of matching domains in PPC. We also observed effects on distinct but related movements (e.glift and grasp) as well as on very different movements involving other body parts. For example, reversibly deactivating a forelimb lift/reach domain in M1 could eliminate a movements in the grasp domain of PPC as well as movements in an unrelated domain such as eye blink. Because projections from M1 to PPC are topographically restricted, our data suggest that the cross-domain effects observed in this investigation are mediated by the dense intrinsic connectivity in PPC. Through these connections, the activity of an M1 domain greatly impacts both related and unrelated PPC domains. Such functional connectivity of disparate movement representations may underlie coding of movement sequences or, through simultaneous activation, may produce a large number of possible combinatorial movements.

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Poster

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Title: Multisensory interactions between the visual and tactile motion systems

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Abstract: We live in a world that requires us to interact with dynamically moving objects of varying sizes and shapes. To function efficiently in this environment it is crucial that inputs from multiple sensory systems merge to form coherent percepts of object representations. While numerous studies have studied how individual features of objects are represented within a sensory system, there are relatively few studies that investigate how features across sensory modalities are combined during object processing and manipulation. Here, we studied the neural mechanisms mediating multisensory integration of motion features between the visual and tactile systems. We used a combined experimental approach by conducting psychophysical experiments in humans and performing single-unit recordings in awake-behaving non-human primates (Macacca mulatta). Tactile stimuli were presented via a custom-made device composed of 400 independently-controlled motors that converged on a 1 cm2 area. This device allowed us to present a wide variety of stimuli such as tactile random dots, sinusoidal gratings and plaids, which were paired with corresponding stimuli in the visual modality. Visual stimuli were presented via a computer monitor that reflected images onto a first-surface mirror, which was spatially aligned to the subject's hand. Psychophysical data showed that visual motion stimuli biased tactile motion perception, and vice versa. The magnitude of the bias was analogous across both sensory modalities. Further, the data indicate that the perceived motion direction of visual and tactile stimuli is predicted by a vector average model composed of the velocity vectors (i.e. speed and direction) of the multisensory stimuli. Finally, single-unit pilot data showed that MT cells encode the direction of motion of tactile stimuli. Taken together, these data indicate that visual and tactile motion perception is mediated by a common set of neural areas and that MT cortex may play a pivotal role within this network.

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Poster

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Title: Spiking activity *in vivo* suggests a slightly sub-critical brain state in rats, cats and monkeys

Authors: ***V. PRIESEMANN**[1,2], M. WIBRAL[3], M. VALDERRAMA[4], R. PRÖPPER[5], M. LE VAN QUYEN[6], J. TRIESCH[7], T. GEISEL[1,2], D. NIKOLIC[8], M. MUNK[9]
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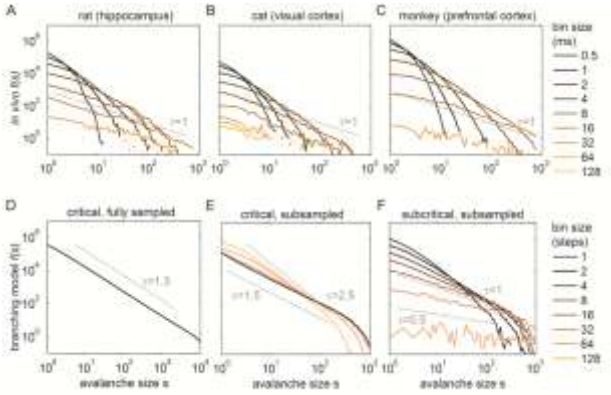
Abstract: Neural activity *in vitro* can show bursts of activity. These bursts are termed neural avalanches and their size distribution *f(s)* approximates a power law [Beggs & Plenz, 2003]. Since power law distributions are characteristic for self-organized critical (SOC) states [Bak, Tang, Wiesenfeld, 1987], neural activity was proposed to be SOC, too. Moreover, SOC may provide a basis for optimal information processing [Shew & Plenz, 2013]. Evidence for the SOC hypothesis has been obtained for coarse measures of neural activity (LFP, EEG, MEG, BOLD), but surprisingly for *spiking* activity *in vivo* evidence for SOC is still missing. Therefore we analyzed highly parallel spike recordings from rats (hippocampus), cats (visual cortex) and monkeys (prefrontal cortex). For all recordings the *f(s)* were similar (Fig. 1 A-C), but showed fundamental differences to *f(s)* from a critical spiking model (Fig. 1 D), even under subsampling

(Fig. 1 E). The differences between *in vivo* dynamics and model dynamics could be overcome by decreasing the model's excitatory synaptic strength, while increasing its external input commensurately (Fig. 1 F). Thereby the model became subcritical and its separation of time scales (STS), which is fundamental to SOC, was eliminated. The match between the subcritical model and the neural activity held for standard and novel avalanche measures (*f(s)*; branching parameter; mean avalanche size; frequency of single events) even when changing the temporal bin size over its full range. In addition, we showed that the same results held for local field potentials recorded in humans. These results suggest that neural activity *in vivo* is not SOC, but instead reflects a slightly subcritical regime without STS. This regime strikes a balance between optimal information processing and the need to avoid runaway activity. In this regime, avalanches are not temporally separated bursts, but form a mélange. Potential advantages of this regime compared to SOC are faster information processing (due to the lack of STS) and keeping a safety margin from supercriticality, which has been linked to epilepsy.



Disclosures: **V. Priesemann:** None. **M. Wibral:** None. **M. Valderrama:** None. **R. Pröpper:** None. **M. Le Van Quyen:** None. **J. Triesch:** None. **T. Geisel:** None. **D. Nikolic:** None. **M. Munk:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 432.15/EE15

Topic: D.03. Multisensory

Support: ANR-11-BSV4-520

ANR-11-LABX-0042

Title: The special role of the claustrum in monkey's interareal cortical communication

Authors: *A. R. RIBEIRO GOMES, C. LAMY, P. MISERY, K. KNOBLAUCH, H. KENNEDY
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Abstract: Although it is well known that cortical areas establish extensive connections with numerous subcortical structures, the potential role of these connections in promoting interareal cortical communication has been largely disregarded, even though some authors suggest that they play an important role in information exchange between cortical areas (Guillery and Sherman 2002; Saalmann, Pinsk et al. 2012). By carrying out 5 paired injections with two highly distinctive retrograde tracers (DY and FsB) in the macaque monkey, we have (1) determined the range of subcortical structures projecting to each injected area and quantified the projections of each; and (2) identified which subcortical structures participate in the formation of cortical-subcortical-cortical loops (CSCLs). CSCLs exist when the afferents to a cortical area X are located in close proximity to the afferents to cortical area Y in a given subcortical structure. Because their formation is expected to be influenced by the distance separating each areas X and Y (Shipp et al., 2003), we have examined CSCLs with respect to two pairs of widely separated areas (7m-10; TEpd-8B) and three pairs of nearby separated areas (V1c-V4c; V4p-V4pc; F2-F7). The subcortical input to the investigated areas ranges between 2% and 11% of the total extrinsic input to these areas; the remaining 98% to 89% originates from the cortex. All target areas receive projections from multiple subcortical sources and the projections' weights of individual subcortical structures overlap those of the values from individual cortical areas. The strongest subcortical projection arises from the claustrum, excepting to V1c which arises from the LGN closely followed by the claustrum. The maximum extent of intermingling of neurons projecting to each areal pair was consistently found in the claustrum. Our findings demonstrate that thalamic CSCLs only contribute to inter-areal communication for nearby area pairs (e.g. V1-V2, V1-V4, F2-F7) and here the band-with is relatively low. More distant area pairs (10-7m, 8B-TEpd) fail to show a significant thalamic CSCL. In contrast to these findings, the claustrum CSCLs exhibit high band-widths for information exchange across areas near and widely separated in the cortex. Hence future large-scale models of the cortical network will have to take these claustrum CSCLs in to account. Our observations will doubtlessly contribute to the present day speculation on claustrum function (Crick and Koch 2005).

Disclosures: A.R. Ribeiro Gomes: None. **C. Lamy:** None. **P. Misery:** None. **K. Knoblauch:** None. **H. Kennedy:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 432.16/EE16

Topic: D.03. Multisensory

Title: Cortical thickness gradients in functional hierarchies

Authors: *K. WAGSTYL[1], L. RONAN[1], S. BEUL[2], P. FLETCHER[1]
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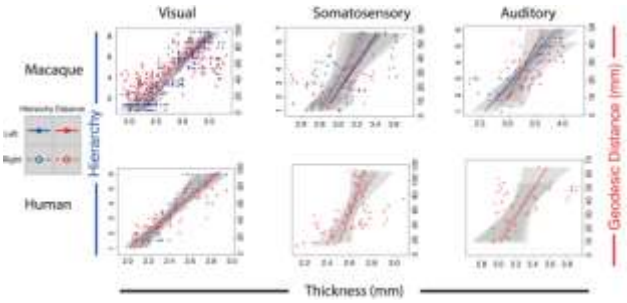
Abstract: The brain processes information through functional hierarchies, where higher functioning regions respond to more varied and complex signals. Histological studies have observed that cortical laminar structure (Barbas 1986) and neuronal density (Cahalane 2012) varies according to these functional demands. In this experiment we investigated whether there is a consistent structural MRI characteristic of functional hierarchies. Specifically we postulated that functional hierarchies may be characterized by an increasing cortical thickness gradient. To test this hypothesis we quantified the cortical thickness of the visual, somatosensory auditory hierarchies using structural MR image data from humans and macaque. Our results demonstrate gradients for each species, suggesting that this may be a generalised feature of functional hierarchies. Cortical reconstructions were generated using structural MRI data from 1 macaque and 83 human subjects using the surface reconstruction software Freesurfer. Cortical thickness was measured and smoothed by random parcellation of the cortex, to minimise differences caused by folding. Regions in the human and macaque hierarchies were parcellated using Caret and literature based hierarchies (Felleman 1991). Geodesic distance from primary sensory region was used as an additional estimate of hierarchical level. Using linear mixed effects modelling, with fixed effects of hemisphere and random effects of individual, there was a significant correlation between thickness and hierarchical level in macaque ($t = 21.1, 4.5 \& 6.9$, $p<0.001$, hierarchical level) and humans ($t = 92.1, 26.3 \& 19$, $p<0.001$, geodesic distance) These gradients result reveals a close coupling between cortical structure and functional demand, enabling further interpretability of morphological measures. Longitudinal analysis of these gradients may offer further insight into the structure-function relationship in normal and atypical neurodevelopment.



Disclosures: **K. Wagstyl:** None. **L. Ronan:** None. **S. Beul:** None. **P. Fletcher:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 432.17/EE17

Topic: D.03. Multisensory

Title: Neuropeptide distribution in the human parabrachial nucleus

Authors: B. GEHRING[1], *S. DE LACALLE[2]
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Abstract: The Parabrachial Nucleus (PB) is a pontine structure composed of several cell groups located around the superior cerebellar peduncle, with a crucial role in autonomic control. In the human brain, the PB is divided into a medial (MPB) and lateral (LPB) nuclei. The human PB is cell poor and does not seem to contain the many subnuclei described in the rat. Nonetheless, there are a number of chemically distinct subdivisions that as well as contributing to establish homologies, may be of value in pathological investigations. Following our earlier work using CGRP as a marker for ascending visceral pathways in the human brain (de Lacalle & Saper, 2000), here we describe the distribution of galanin (GAL-), substance P (SP-) and neurotensin (NT-) immunoreactive elements. Our observations were made on horizontal 50µm thick sections through the brainstem from 3 neurologically normal individuals, obtained at routine autopsy. Tissue was processed for immunocytochemistry using commercially available antibodies. We found several areas of dense peptide immunoreactivity in fibers, as well as scattered stained cell bodies. The distribution of peptide-stained fibers was strikingly conserved compared with that described in the rat. GAL-ir fibers were present in both subdivisions, but more abundant in the LPB than the MPB, with their distribution somewhat overlapping that of CGRP-ir fibers. By

contrast, NT-ir fibers were more prominent in the MPB than in the LPB. SP-ir fibers were found in both MPB and LPB, with a dense cluster of fibers in the rostral region, and scattered SP-ir neuronal profiles throughout the area between the locus coeruleus and the MPB. Compared with the literature on the functional anatomy of the PB and its afferent and efferent projections, our results suggest that these neuropeptides found in distinct areas within the human PB may also provide chemical coding for the relay of specific visceral information.

Disclosures: B. Gehring: None. **S. de Lacalle:** None.

Poster

432. Multisensory: Neural Circuitry and Connections

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Program#/Poster#: 432.18/EE18

Topic: D.03. Multisensory

Support: NSFC 91132702

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NSFC 91132702

NSFC 2014CB846101

Title: A decentralized architecture for neural information integration

Authors: W. H. ZHANG[1,3], *M. J. RASCH[1,2], S. WU[1,2]
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Abstract: Understanding information integration in the brain is of critical importance. It was found that the brain combines information from different modalities in an optimal way as predicted by Bayesian inference. For example, while walking, the visual and vestibular signals both carry useful information of motion direction, which can be seamlessly fused in the brain to get a more reliable estimation of the motion direction than either of the inputs could deliver on its own. How exactly brain areas integrate multisensory information optimally remains a challenge. The general view implicitly assumes that one determined centralized multisensory area receives feed-forward inputs from different modalities to integrate all information.

However, optimal information integration in such a central area was only shown to exist if important nonlinear neuronal properties and recurrent neural connectivity are neglected. Inspired by ideas from engineering, we here propose a conceptually novel architecture for implementing optimal information integration in neural circuits. We show that optimal information integration can be achieved in a decentralized manner using reciprocally connected modular processors (i.e., cortical regions). In such a decentralized architecture, no brain area is in the center of system topology, so that its robustness to local failures is markedly improved. Moreover, information integration in a decentralized system can be understood as an emergent and collective behavior of all reciprocally connected processors. As a result, optimally combined multisensory information is made locally available in each individual cortical region without the need of further distribution. Using biologically realistic neural networks, we build a decentralized information integration system that replicates experimentally observed Bayesian optimal integration behaviors. Our model not only delivers a natural explanation of the widely observed reciprocal connectivity between cortical regions, but also provides a novel framework of how optimal information integration might be achieved in neural circuitry.

Disclosures: **W.H. Zhang:** None. **M.J. Rasch:** None. **S. Wu:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.01/EE19

Topic: D.04. Vision

Support: NSF Grant DGE 1256260

Title: Optimal stimuli for evoking melanopsin-based photoresponses in intrinsically photosensitive retinal ganglion cells

Authors: ***O. WALCH**[1], L. ZHANG[2], D. FORGER[1], K. WONG[2]
[1]Mathematics, [2]Ophthalmology and Visual Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) are capable of responding directly to light through the photopigment melanopsin, although they also receive synaptic input from rod and cone photoreceptors. Their discovery has prompted a significant body of work on many aspects of their physiology, including morphological subtypes, electrical behavior, and phototransduction pathway. Of interest for a number of reasons, including their role in

controlling the pupillary light reflex and other forms of subconscious vision, ipRGCs are particularly relevant to circadian research as they are responsible for entraining the circadian clock to light-dark cycles. The light-evoked responses of ipRGCs are far more sustained than those of conventional ganglion cells, and can stably encode absolute light intensity for many hours. These physiological differences suggest that the role of ipRGCs is to report ambient luminance, rather than to capture accurate spatio-temporal dynamics in the visual field. Mathematically modeling the process by which light is converted into signal in ipRGCs can lend valuable insight to their underlying physiology. Previous modeling work on the phototransduction pathway in ipRGCs has focused on the spectral responses of cells, framing much of the model equations through the lens of action spectra. Here we take a more biochemical approach, using differential equations to fit a model of the molecular interactions that compose the melanopsin phototransduction cascade of a generic ipRGC to multielectrode-array data. Using techniques for the calculus of variations, we then make optimality predictions for evoking maximal firing response from ipRGCs for several functional forms of light dependence.

Disclosures: O. Walch: None. **L. Zhang:** None. **D. Forger:** None. **K. Wong:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.02/EE20

Topic: D.04. Vision

Support: EY000450

Title: Maturation of the mouse rod photoreceptor

Authors: *D. T. WHITAKER[1,2], K. KOORAGAYALA[1], C. CAMPLA[1,3], K. MOLLURA[1], J. COOKE[1], A. SWAROOP[1]
[1]Natl. Eye Inst., NIH, Bethesda, MD; [2]Neurosci., Texas A&M Univ., College Station, TX; [3]Oxford Univ., Oxford, United Kingdom

Abstract: The universe we live is a beautiful and wondrous place. To gather a sense of its grandeur, we use our sense of sight (vision). Not only does this sense provide us with a sense of the grandness of our environment, it affects our and other organisms' survival, behavior and emotions. Much is known about the visual system, specifically the retina, but as a whole we are

still just scratching the surface of what is there is left to discover. Our group focuses almost exclusively on the rod photoreceptors during development through aging and degeneration. We have gathered a substantial body of knowledge of the transcriptome of these cells in each stage of its life; in addition, we know that NRL is the master regulator driving the progenitor towards the rod photoreceptor cell fate. Less has been uncovered describing the roles that NRL's transcriptional targets play during retinal development or within the mature retinal environment. Here, we use the previously gathered developmental transcriptome and targetome (from NRL ChIP-sequencing) to identify important agents in various processes of this cell's development. To accomplish this goal, we have performed an extensive screen in which individual NRL targets have been knocked down *in vivo* and the resulting mature phenotype assessed. This screen allows us to begin to identify the extent of NRL's targets' roles in rod photoreceptor development as well as to start assessing the role of many of these genes in processes important for the retinal and neuroscience communities as a whole.

Disclosures: D.T. Whitaker: None. **A. Swaroop:** None. **C. Campla:** None. **K. Kooragayala:** None. **K. Mollura:** None. **J. Cooke:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.03/EE21

Topic: D.04. Vision

Title: Discovery of small molecule melanopsin antagonists using a high-throughput screening assay

Authors: *H. ZHONG[1], L. YUAN[1], J. SPROUSE[2], R. ARTYMYSHYN[3], S.-P. HONG[3], M. MARZABADI[3], K. JONES[3]
[1]U-Pharm Labs. LLC, Parsippany, NJ; [2]Cyanaptic LLC, Stonington, CT; [3]Lundbeck Res. USA Inc, Paramus, NJ

Abstract: The photopigment melanopsin (Opn4) is a G protein-coupled photoreceptor that is exclusively expressed in retinal ganglion cells. Melanopsin, different from rod and cone photoreceptors, mediates behavioral adaptation to ambient light and other non-image-forming photic responses, such as photophobia, circadian rhythms and neuroendocrine function. Pharmacological agents that specifically modulate melanopsin signaling might be useful for investigating these light-dependent physiological mechanisms and diseases. Here we report the

establishment of a high-throughput screening assay for the discovery of melanopsin compounds. cDNA for the human melanopsin receptor was cloned and a stable line in Chinese hamster ovary (CHO) cells was developed for its expression. The activation of Gq-coupled melanopsin in CHO cells by light induced different calcium mobilization kinetics depending on whether or not the cells were pre-incubated with 9-cis-retinal. A search for small-molecule antagonists was established using the Hamamatsu FDSS 6000/7000 that has a high-throughput screening capability for G protein coupled receptors (GPCRs). The screen against a library of 80,000 compounds yielded multiple compounds belonging to a sulfonamide structural scaffold. Chemical optimization resulted in specific and potent (nM) melanopsin antagonists free of significant activity (<50% binding activity at 10 μM) against a panel of 74 targets including GPCRs, ion channels and enzymes. When dosed systemically, one of these compounds displayed significant exposure (μM) in mouse brain and in rat brain and retina. The discovery of novel antagonists provides the first step in the development of specific modulators of the melanopsin system for the study of light-dependent behavior and physiology.

Disclosures: **H. Zhong:** A. Employment/Salary (full or part-time):; U-Pharm Laboratories LLC, Cyanaptic LLC, Lundbeck Research USA Inc. **L. Yuan:** None. **J. Sprouse:** None. **R. Artymyshyn:** None. **S. Hong:** None. **M. Marzabadi:** None. **K. Jones:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.04/EE22

Topic: D.04. Vision

Support: NIH Grant EY 013865

Macula Vision Research Foundation

Title: IGF1 and PMA act through specific phoshatases to induce rod photoreceptor differentiation

Authors: ***P. T. BROWN**, C. PINZON-GUZMAN, T. XING, C. J. BARNSTABLE
Neural and Behavioral Sci., Penn State Univ. Col. of Med., Hershey, PA

Abstract: **Introduction:** In mice the transition of a cell from neural progenitor to rod precursor is controlled by several transcription factors. Changes in the expression of these transcription

factors within the first few postnatal days drives cells to a mature rather than a progenitor phenotype. We have shown previously that this process is sensitive to manipulation by cytokines and growth factors. Dephosphorylation of pSTAT3 is a necessary step in the transition into a terminally differentiated rod and treatment with CNTF blocks rod development through stimulation of pSTAT3. Conversely, treatment with IGF1 or the PKC activator PMA increases rod photoreceptor differentiation through two PKC isoforms – β1 and γ. The purpose of this work was to determine the pathways downstream of PKC that IGF1 and PMA modulate to induce rod differentiation. **Methods:** Animals were treated in accordance with IACUC and Pennsylvania State University CoM guidelines. Postnatal day 1 retinas were taken from C57BL/6 mice for western blots or immunohistochemistry. Tissues for western blots were treated with inhibitor for 90 min with IGF1 or PMA added for the last 30 minutes. Retinal explants used for immunohistochemistry were treated with inhibitor and IGF1 or PMA for 4 days before fixing, sectioning, and labeling. **Results:** Sodium orthovanadate inhibits the activity of a wide range of phosphatases. Pretreating retinas with sodium orthovandate prior to incubation with IGF1 or PMA prevented the decrease of pSTAT3 caused by either treatment. PTEN was identified as a potential phosphatase regulating these events. Retinas treated with the PTEN inhibitor bpV and IGF1 or PMA produced fewer rods than retinas treated with IGF1 or PMA alone, but not as few as control retinas. Treatment with bpV did not alter the PMA-mediated decrease of pSTAT3. The role of several protein tyrosine phosphatases was tested using NSC87877, an inhibitor that blocks the activity of seven phosphatases at high concentrations and only Shp1/2 at low concentrations. Both concentrations diminished the IGF1- and PMA-induced increase of rod photoreceptor differentiation back to control levels and blocked pSTAT3 dephosphorylation by IGF1 and PMA. **Conclusions:** Here we show that IGF1 and PMA affect phosphatase activity to induce rod photoreceptor differentiation. Firstly, IGF1 and PMA enhance PTEN activity to increase rod production; however, PTEN does not dephosphorylate pSTAT3. It likely acts upstream of STAT3, perhaps on PIP3. Secondly, IGF1 and PMA activate a protein tyrosine phosphatase inhibited by NSC87877. The low concentration is as effective as the high concentration, suggesting that Shp1/2 dephosphorylates pSTAT3.

Disclosures: P.T. Brown: None. **C. Pinzon-Guzman:** None. **T. Xing:** None. **C.J. Barnstable:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.05/EE23

Topic: D.04. Vision

Support: KAKENHI 26780415

Grant for Tateisi Science and Technology Foundation

Title: Behaviorally measured visual contrast sensitivity of Royal College of Surgeons Rats and its temporal loss

Authors: ***S. SOMA**, N. SUEMATSU, S. SHIMEGI
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Abstract: The Royal College of Surgeons (RCS) rat is the animal model of retinal degeneration. The inability of the retinal pigment epithelial cell causes a progressive loss of rod and cone photoreceptors, which occurs primarily in the first few months of life. Using this rat model of retinitis pigmentosa, the various recovery studies including the graft study and development of artificial vision systems have been conducted. Moreover, the visual ability of RCS rat has been examined by recording the visual responses in the superior colliculus, showing the temporal loss of the visual responses. However, the visual ability in the freely behaving RCS rat have not been psychophysically measured yet because their visual function is lost before they learn the visual stimulus detection task which generally takes a long-term training period. Therefore, the temporal changes in behavioral visual sensitivity of RCS rat remain unknown. To overcome this problem, we developed an efficient and stable training system for the two-alternative forced-choice visual cue detection task (2AFC-VCDT) for freely behaving rodents (using pigmented Long-Evans rats). To facilitate the task learning, we introduced a spout-lever as the operandum and a three-step training program with four ingenuities: 1) a salient stimulus to draw passive attention, 2) a reward-guaranteed trial to keep motivation, 3) a behavior-corrective trial, and 4) switching from a reward-guaranteed trial to a non-guaranteed one to correct behavioral patterns. Our new training system realizes one-week completion of the whole learning process, during which all rats were able to learn effortlessly the association between 1) lever-manipulation and reward and 2) visual stimulus and reward in a step-by-step manner. Thus, our new system provides an effective and stable training method for the 2AFC-VCDT. Using this method, we trained RCS rats before development of retinitis pigmentosa at 3 weeks of age, and successfully measured their visual contrast sensitivity. The RCS rats showed the lower contrast sensitivity function than Long-Evans rats, which declined over the three months of life.

Disclosures: **S. Soma:** None. **N. Suematsu:** None. **S. Shimegi:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.06/EE24

Topic: D.04. Vision

Title: Melanopsin - a possible trigger of lateralization in pigeons?

Authors: *R. KLOSE, F. STROCKENS, O. GUNTURKUN
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Abstract: Functional and structural asymmetries of the nervous system are a common feature of vertebrates. However, it is unknown if lateralization is mainly determined by genetic factors or triggered by environmental factors. There is strong evidence for an involvement of the environmental factor light in the induction of asymmetries of the visual system in pigeons. This dominance is caused by an asymmetric position of the pigeon embryo inside the egg, with the right eye being exposed to stronger light stimulation than the left one. This lateralized stimulation induces the development of an asymmetrical visual system afflicting visual-guided behavior in the adult bird. However, it is unclear how such light stimulation can induce lateralization, since classical photoreceptors which normally detect light in adult animals are not functionally expressed in the embryonic retina before hatch. A possible explanation could be given by photosensitive molecules that have been shown to be present at an early stage during embryonic development in retinal ganglion cells (RGCs) of other animals. RGCs have functional connections to primary visual areas already before hatch and would, therefore, be an ideal location for a potential light-trigger. A possible candidate for an early photosensitive instance within RGCs is the molecule Melanopsin, a non-image forming photoreceptor. Studies in different vertebrates show, that Melanopsin is expressed in RGCs, as well as in the inner nuclear layer. We therefore assume that Melanopsin could be a valid candidate for triggering lateralization in pigeons. To support this hypothesis we first sequenced the so far unknown Melanopsin gene of the pigeon and subsequently stained Melanopsin in the retina by in-situ hybridization (ISH) and immunohistochemical staining (IHC). We discovered that the pigeon's Melanopsin gene codes for at least two isoforms and reveals large similarities to the Melanopsin sequence of other birds. In addition it also shows concordance to mammals, as well as to reptiles, amphibians and fish. The ISH and IHC in adult pigeons show a high expression of Melanopsin in ganglion cells and lower expression in the inner nuclear layer. Our results supply for the first time the complete pigeons Melanopsin gene sequence as well as data about the distribution inside the adult retina of pigeons. We currently plan to stain embryonic and post-hatch retinae, as well as run a Calcium-Imaging study to show the light dependent-activation of pigeon Melanopsin. This could deliver a further proof for the involvement of Melanopsin in the induction of lateralization in pigeons.

Disclosures: **R. Klose:** None. **F. Strockens:** None. **O. Gunturkun:** None.

Poster

433. Photoreceptors

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.07/EE25

Topic: D.04. Vision

Support: NIH grant EY019053

Title: The role of melanopsin phosphorylation in mouse behavior and physiology

Authors: P. SOMASUNDARAM, *P. ROBINSON
Biol. Sci., UMBC, Baltimore, MD

Abstract: Melanopsin is a unique visual pigment, expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) of mammalian retina. It is a G-protein coupled receptor (GPCR) involved in photoreception for non-visual functions such as circadian photo-entrainment and pupillary light reflex. Recent work demonstrates that melanopsin is capable of rescuing defects in simple visual tasks. This calls for a broader understanding of the kinetics and regulation of melanopsin activity. Melanopsin structure and *in vitro* data suggest that the activity of this visual pigment is regulated by phosphorylation of the receptor by a G-protein coupled receptor kinase (GRK) and a protein kinase A (PKA) in light and dopamine dependent manners respectively. The working hypotheses for this project are that GRK mediated melanopsin phosphorylation controls the lifetime of the active protein by initiating deactivation of the receptor, and that PKA mediated phosphorylation contributes to ipRGC adaptation by modulating the activity of the receptor. I aim to demonstrate the impact of these phosphorylations on physiology and behavior of mice. Melanopsin genes (*Opn4*) carrying targeted mutations of either GRK or PKA phosphorylation sites have been incorporated in the reverse orientation between loxP sites into an adeno-associated viral vector backbone, thus rendering them dependent on Cre-loxP recombination for expression. A viral based gene delivery approach will used to introduce these floxed constructs into $Opn4^{Cre/Cre}$ mice to generate transgenic mouse models. Packaging of the floxed constructs into suitable AAV2 capsids is currently underway. These transgenic mice are expected to show abnormal circadian behavior and pupillary light reflex, and a deficit in ipRGC adaptation. This research could potentially demonstrate a previously unexplained mechanism underlying ipRGC photoreceptor adaptation and establish the significance of phosphorylation on organismal behavior in the context of non-image forming vision.

Disclosures: P. Somasundaram: None. **P. Robinson:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.08/EE26

Topic: D.04. Vision

Support: UAB Department of Ophthalmology

Title: Cholinergic and melanopsin dendritic stratification in the inner plexiform layer of the macaque retina

Authors: *K. Q. CHANG[1], C. E. STRANG[2], P. D. GAMLIN[3]
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Abstract: In the primate, intrinsically photosensitive retinal ganglion cells (ipRGC) express melanopsin and project to central targets involved in both image and non-image-forming visual processing (Hannibal et al., 2014). To better characterize the ipRGCs in primate retina, we investigated the distribution and relationship of their dendritic stratification to the well-defined choline acetyltransferase (ChAT) dendritic labeling within the inner plexiform layer (IPL). Immunohistochemistry was performed on both whole-mount and 10μm cryostat sectioned macaque retinas. Indirect immunofluorescent staining for melanopsin was performed using an affinity-purified, custom polyclonal antibody created against a 19 amino acid residue peptide, MNPPSGPRVPPSPTQEPSC, from the N terminus of the macaque melanopsin protein, and validated by western blot analysis. Immunostaining for cholinergic processes was performed with a well-described antibody against ChAT (AB1440P, Chemicon). In addition, Hoescht counter staining was used. As previously described, ChAT immunoreactive (IR) processes formed two narrow, well-defined layers in the IPL, with one layer in sublamina a and the other in sublamina b (Rodieck and Marshak, 1992; Yamada et al, 2003). As previously described, melanopsin-IR soma were located in both the inner nuclear layer and ganglion cell layer. The processes of the ipRGCs stratified in two distinct layers in the IPL. One layer was localized at the border of sublamina a close to the inner nuclear layer where partial co-stratification with ChAT-IR processes was observed. The other layer was localized at the border of sublamina b close to the ganglion cell layer. The stratification patterns suggest that the processes of several populations of ipRGCs could receive cholinergic inputs.

Disclosures: K.Q. Chang: None. **C.E. Strang:** None. **P.D. Gamlin:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.09/EE27

Topic: D.04. Vision

Support: NSC 101-2311-B-002-023

Title: Determining the developmental lineage of novel photoreceptors that control circadian rhythms

Authors: *H.-M. WANG, S.-K. CHEN
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Abstract: Since the discovery of melanopsin, the novel photoreceptor, melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs), has been shown to participate in many non-image forming functions such as circadian rhythms and pupillary light reflex. While most ipRGCs express brn3b (POU4F2), the general transcription factor for retinal ganglion cells development, a portion of ipRGCs do not express brn3b and dominantly innervate suprachiasmatic nucleus (SCN), which is the central clock for circadian rhythm in mammals. In retinal development, different time points of cell differentiation strongly imply different cell types. To identify differentiation time point of brn3b negative ipRGCs, we used 5-ethynyl-2'-deoxyuridine (EdU) and melanopsin immunostaining to label mitotic ipRGCs at specific embryonic stage in wild type mice and transgenic mice without brn3b-expressing ipRGCs. Our data show that brn3b negative ipRGCs derive from retinal progenitor cells in a very short period while the whole population of ipRGCs arised in relatively long period. It strongly suggests that SCN-innervating brn3b negative ipRGCs are a distinct sub-population of ipRGCs, which provides new insight on future investigation of circadian rhythms and retina.

Disclosures: H. Wang: None. **S. Chen:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.10/EE28

Topic: D.04. Vision

Title: Electrophysiological investigation of the compound eye of the brown marmorated stink bug

Authors: N. S. ARNOLD[1], V. D. C. SHIELDS[1], T. HEINBOCKEL[2], *A. B. LALL[3]
[1]Biol. Sci., Towson Univ., Towson, MD; [2]Anat., Howard Univ. Col. of Med., Washington, DC; [3]Dept. of Biol., Howard Univ., Washington, DC

Abstract: The brown marmorated stink bug, *Halyomorpha halys,* is an invasive species that was introduced to North America more than twenty years ago. Through the years, it has become a nuisance pest. It is responsible for damage and loss to a wide variety of fruit and vegetable crops that it feeds on. More recent control methods have incorporated pesticides, in addition to olfactory and, to a much lesser extent, visual traps. As this species is positively phototoxic, our research focus was to describe the characteristics of the visual system of this insect, which consist of a pair of compound eyes and two simple ocelli. Electroretinograms (ERGs) were obtained by inserting a glass electrode into the corneal surface of the eye using a conventional ERG recording method. Several findings were obtained. (1) The electrical response was a monophasic corneal negative wave consisting of an initial phasic wave followed by a maintained component for the duration of the flash. There was no off-transient at the termination of the light stimulus. (2) V-log I curves were obtained over six log units of change in stimulus intensity during the photo phase. (3) The latency of the response shortened as a function of an increase in the intensity of the flash from ~40 msec in dim light stimuli to ~10 msec in bright light stimuli. (4) The eye had a flicker fusion frequency above 30 flashes/second. The stink bug is a diurnal insect.

Disclosures: N.S. Arnold: None. **V.D.C. Shields:** None. **T. Heinbockel:** None. **A.B. Lall:** None.

Poster

433. Photoreceptors

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Program#/Poster#: 433.11/FF1

Topic: D.04. Vision

Support: Grant 8KL2TR000112-05 from the National Center For Advancing Translational Sciences (NIH)

Sigma Xi Grant-in-Aid of Research (GIAR) Award

Title: Acetylcholine-mediated stimulation of retinal ganglion cell photoreceptors

Authors: *P. SODHI, A. T. E. HARTWICK
Ohio State Univ., Columbus, OH

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) are photoreceptors involved in non-image forming visual processes such as circadian rhythms and the pupillary light reflex. Light information carried by ipRGCs also influences mood, cognition, and alertness. Invertebrate phototransduction is initiated by Gq-protein-coupled phospholipase C (PLC) signaling. Evidence indicates a similar pathway for ipRGC phototransduction, consistent with the strong homology between invertebrate opsins and melanopsin (the ipRGC photopigment). Acetylcholine (ACh), a neurotransmitter released from starburst amacrine cells in the mammalian retina, can activate the Gq-PLC pathway through metabotropic muscarnic receptors. Our aim was to determine whether acetylcholine can directly stimulate ipRGCs through a muscarinic acetylcholine receptor (mAChR)-mediated Gq-coupled PLC signaling pathway. Retinas were dissected from adult and neonatal (P8-P13) Long Evans rats and placed RGC-side down on a multi-electrode array while being continuously superfused with heated, oxygenated Ames medium. Each retina was stimulated with 20 sec pulses of blue light (470 nm; 2x1014 photons/s/cm2) and ipRGCs were identified based on their light responses in the absence of rod/cone-driven synaptic input. The retinas were exposed to compounds known to directly stimulate cholinergic receptors (100μM carbachol) or raise endogenous Ach levels (20μM physostigmine) in the presence or absence of mAChR (20μM atropine) and nAChR (100μM tubocurarine) antagonists. In neonatal and adult ipRGCs, treatment with either carbachol or physostigmine significantly ($p<.001$) increased the duration and total number of spikes fired as compared to ipRGC responses before and after treatment. Application of mAChR antagonist atropine ($p<.001$), but not nAChR antagonist tubocurarine, reversed the effects of carbachol and physostigmine. This study enhances our understanding of G-protein signaling pathways that influence mammalian ipRGC responses and their contribution to pupil size. The significance of this work is that it identifies a novel pathway for activating ipRGCs, that is separate from light-driven activation of the melanopsin photopigment contained by these cells. This pathway could be targeted by pharmacologic drugs to influence non-visual functions (i.e. alertness and cognition) typically regulated by the amount of light captured by these cells. It also presents a therapeutic approach for correcting conditions in which ipRGC stimulation may be abnormal, such as seasonal affective disorder.

Disclosures: P. Sodhi: None. **A.T.E. Hartwick:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.12/FF2

Topic: D.04. Vision

Support: BBSRC fellowship BB/I017836/1

Title: Colour-opponent twilight coding regulates the mammalian circadian clock

Authors: *T. BROWN, L. WALMSLEY, L. HANNA, J. MOULAND, F. MARTIAL, A. WEBB, D. BECHTOLD, R. LUCAS
Univ. of Manchester, Manchester, United Kingdom

Abstract: It is well established that the daily solar cycle is the dominant environmental signal regulating mammalian circadian entrainment. However, the specific features of the light environment that are most important for photoentrainment remain incompletely understood. In particular, the extent to which changes in the spectral composition of ambient illumination could provide important timing cues remains unknown. Here we investigated this possibility using a combination of computational modelling, electrophysiological recordings and behavioural assays of circadian entrainment in mice. First, using spectral irradiance measurements taken across multiple solar cycles, we show that relative spectral composition is more predictive of solar angles around twilight than global measures of irradiance. We go on to show, using *in vivo* electrophysiological recordings, that a subset (~20%) of SCN neurons exhibit the spectrally-opponent responses required to extract this information. Further, using visual stimuli designed to recreate various stages of twilight for the mouse visual system, we show that the firing rates of spectrally-opponent SCN cells reliably track solar angles around twilight (a property that is deficient in non-opponent SCN cells). Finally, using assays of photoentrainment under simulated twilight, we show that this opponent mechanism significantly influences circadian phase, allowing mice to appropriately time their activity to the middle of the night. Together, these findings reveal an important and hitherto unrecognised aspect of the sensory inputs that drive circadian photoentrainment.

Disclosures: T. Brown: None. **L. Walmsley:** None. **L. Hanna:** None. **J. Mouland:** None. **F. Martial:** None. **A. Webb:** None. **D. Bechtold:** None. **R. Lucas:** None.

Poster

433. Photoreceptors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 433.13/FF3

Topic: D.04. Vision

Title: Detection of vitamin A in the iris of the turtle supports a functional melanopsin

Authors: *J. R. DEARWORTH, Jr., M. S. D'SOUZA, J. SHERMA, M. J. CHEJLAVA
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Abstract: The iris in the turtle (*Trachemys scripta elegans*) is intrinsically photosensitive constricting the pupil when exposed to light. One of the photopigments hypothesized to be involved is melanopsin harboring a vitamin A-type chromophore. Our purpose was to detect for vitamin A compounds and determine the type that could be associated with melanopsin. Tissues were dissected from turtles, homogenized, and reacted with hydroxylamine. Extraction was done by water/methanol/dichloromethane, and extracts then analyzed by high-performance thin-layer chromatography (HPTLC) using cyclohexane/toluene/ethyl acetate (5/3/2, v/v/v) mobile phase on channelled preadsorbent silica gel glass plates with fluorescent indicator (Analtech, catalog #61927). Compounds separated on plates were identified by their retardation factor (Rf) values compared to standards under both white and ultraviolet (254 nm) light. To confirm identity and further discriminate compounds as A_1 or A_2, extracts also were examined by spectrophotometry after reacting with antimony chloride (Carr Price test). Retina possessed two clusters of compounds. A slow cluster was detected with a central Rf=0.37 within the range of isomers for retinaldehyde (A_1) and 3,4-dedihydroretinaldehyde (A_2) oximes, including where the 11-cis oximes derived from a melanopsin-bound form of a chromophore would be expected. A faster cluster (Rf=0.74) was also detected for retina approximating the values for vitamin A_1 and A_2 esters. Iris extracts run on plates showed no detectable clusters; however, when Carr Price tests were done, the iris showed primary λ_{max} at 597 nm matching the peak for all-*trans*-3,4-dedihydroretinaldehyde (A_1) oximes. A similar peak was also measured for pineal. In contrast, Carr Price tests on the retina extracts showed primary λ_{max} absorbance at 692 nm matching the peak generated from all-*trans*-3,4-dedihydroretinaldehyde (A_2) oximes. Results suggest that the melanopsin in the retina harbors a vitamin A_2 chromophore whereas in the iris melanopsin may harbor a vitamin A_1 chromophore. Association with different vitamin A chromophore types for melanopsins in the retina and iris of the turtle support evolutionary functional divergence for controlling pupillary constrictions to light in non-mammals.

Disclosures: **J.R. Dearworth:** None. **M.S. D'souza:** None. **J. Sherma:** None. **M.J. Chejlava:** None.

Poster

433. Photoreceptors

Location: Halls A-C

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Topic: D.04. Vision

Support: NIH R01 EY017452

NIH R21 EY023339

Postdoctoral Research Award, College of Veterinary Medicine and Biomedical Sciences, TAMU

Title: Peptide Lv augments the L-type voltage-gated calcium channels through vascular endothelial growth factor receptor 2 (VEGFR2) signaling

Authors: L. SHI[1], S. KO[2], M. L. KO[1], *G. Y.-P. KO[1]
[1]Vet. Integrative Biosci., Texas A&M Univ., College Station, TX; [2]Med., Baylor Col. of Med., Houston, TX

Abstract: Peptide hormones and growth factors regulate diverse physiological conditions and behaviors in both neural and cardiovascular systems, such as sleep, circadian rhythm, learning and memory, heart rate, cardiac contraction, and blood vessel formation. Previously, we identified peptide Lv, a novel putative peptide that enhances the activity of L-type voltage-gated calcium channels (L-VGCCs) in cone photoreceptors and cardiomyocytes. A proteomics approach to investigate the specific receptors and binding partners of peptide Lv indicated that vascular endothelial growth factor receptor 2 (VEGFR2) was able to interact with peptide Lv. VEGFR signaling is essential for the development of the cardiovascular system in embryos and the formation of new blood vessels in adults. Treatment with peptide Lv in embryonic cardiomyocytes stimulated the tyrosine autophosphorylation of VEGFR2, as well as the activation of downstream signaling including PKC and ERK. Its activity was blocked by the VEGFR2 specific blocker DMH4, but not by SCH202676, an allosteric inhibitor of G protein coupled receptor (GPCR), suggesting that VEGFR2 mediates peptide Lv signaling in cardiomyocytes. Inhibition of VEGFR-tyrosine kinase, PKC, or ERK abolished the peptide Lv

elicited augmentation of L-VGCCs. The voltage-dependent calcium (Ca2+) entry through L-VGCCs promotes various Ca2+-dependent cellular processes and regulates intracellular Ca2+ in both nervous and cardiovascular systems. Hence, our work suggests that peptide Lv might serve as a novel activator of VEGFR2 and regulate VEGFR2 mediated physiological function in retinal photoreceptors and the cardiovascular system. This work was supported by R01EY017452 and R21 EY023339 from the National Eye Institute of the National Institutes of Health to GK, and a postdoctoral research award from the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University to LS. We thank Dr. William Russell, Laboratory for Biological Mass Spectrometry at Texas A&M University for their technical support.

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Poster

433. Photoreceptors

Location: Halls A-C

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Program#/Poster#: 433.15/FF5

Topic: D.04. Vision

Support: E-Rare 2 – SAU/0001/2008

E-Rare4/0001/2012

CENTRO-07-ST24-FEDER-00205 From molecules to man

PEst-C/ SAU/UI3282/2013

Title: Evidence for structural and functional reorganization of visual retinotopic and high level regions in a human model of genetically determined peripheral visual loss

Authors: *M. CASTELO-BRANCO[1], S. FERREIRA[1], A. PEREIRA[1], B. QUENDERA[1], C. MATEUS[1], M. D. R. ALMEIDA[2], E. SILVA[3]
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Abstract: Retinitis Pigmentosa (RP) is an inherited retinal disease characterized by progressive degeneration of photoreceptors. It provides a model to study visual cortical plasticity, because in

contrast with previous studies, which have mainly covered central vision loss, it involves early progressive loss of peripheral vision. We aimed to determine the influence of peripheral loss on visual cortical reorganization using structural and functional MRI. Brain images of 5 RP subjects (49.60±10.60 years; disease duration 30.40±14.29 years) and 10 age- and gender-matched healthy controls were acquired with a 3T scanner and analyzed with BrainVoyager® (linear correlation maps for retinotopy, general linear models, with false discovery rate correction with $p<0.05$, and cortical thickness measures). Freesurfer was used to compute the volume of subcortical structures. First, standard retinotopic mapping (eccentricity and polar angle) was applied to delineate individual visual cortical areas. The second fMRI stimulus consisted in a random sequence of two checkerboard rings (presented at central and paracentral visual fields), during passive viewing and an one-back visual memory task. Stimuli were presented monocularly. RP patients presented a significant reduction in retinal thickness (as measured by optical coherence tomography), bilateral visual fields and visual acuity, consistent with long term photoreceptor loss. In this group, we found evidence for retinotopic reorganization with posterior-to-anterior shift of cortical activation in particular for more central rings. Our measure of reorganization (distance of the center of the map to the occipital pole) was correlated with disease duration for Rings 1 and 2 [$p<0.05$, Spearman's rank correlation coefficient]. When contrasting retinotopic activation under active (one-back task) vs. passive viewing we found that RP participants showed significantly increased activation in area V3 dorsal, suggesting top down modulation of higher level regions. This interpretation is consistent with the results of structural analysis which showed increases in cortical thickness in Brodman Area 7, which is involved in attention processing. Importantly, cortical thickness was decreased in dorsal visual area V3 for RP subjects. Interestingly we found a significant increase in the volume of the caudate nucleus bilaterally ($p<0.005$), which is involved in goal directed attentional navigation. These results suggest structural and functional reorganization of striate and extrastriate cortices of RP patients, possibly caused by feedback signals from higher order cortical areas involved in attention.

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Poster

433. Photoreceptors

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Program#/Poster#: 433.16/FF6

Topic: D.04. Vision

Support: NSC Grant (102-2320-B-030-007) Taipei, Taiwan

Title: HSP27 alters retinal cell existence in a light-induced retinal degenerative model

Authors: *Y.-J. LEE[1], C.-J. HUANG[2,1], Y.-C. CHENG[2], L.-T. TIEN[1], C.-Y. KE[1], C.-C. CHIEN[3,1]
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Abstract: Retinal degeneration is the common cause of vision loss. Some treatments including gene therapy and growth factor injections have been developed to delay or prevent the loss of retinal cells. In this study, we established a light-induced retinal degenerative animal model and found both outer nuclear layer of retina and retinal function, examined by electroretinogram, decreased gradually after 1-week light exposure. We also constructed a viral vector with a gene to silence the expression of heat shock protein 27 (HSP27), and injected the vector into the injured retina. It was found that the histological change of retina correlative to the expression of HSP27 in retinas after light exposure. We also noticed that the expression of HSP27 was detected in the inner plexiform layer, the outer plexiform layer, and the outer segment area in the retinas after one week light expose. The expression of HSP27 was found only detectable in the outer segment area 2 weeks later in the sham-operative animals. In addition, there were more retinal cells preserved in the silent-HSP27 viral injected retina, compared to the sham-operated eyes two weeks after viral injection. It was later found that both the gene and protein expressions of photoreceptor cell marker increases in the retinas received silent-HSP27 viral injection, compared to light only preparations. Silencing of HSP27 does not affect the expressions of ganglion cell marker and amacrine cell marker. We therefore assumed that HSP27 plays an important role on protecting photoreceptors from light-exposed retinal degeneration.

Disclosures: **Y. Lee:** None. **C. Huang:** None. **Y. Cheng:** None. **L. Tien:** None. **C. Ke:** None. **C. Chien:** None.

Poster

433. Photoreceptors

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Topic: D.04. Vision

Support: NSERC Operating to WTA

NSERC Studentship to APO

CIHR Studentship to MGD

Title: Cone photoreceptor regeneration following cone-specific ablation

Authors: ***W. T. ALLISON**, A. P. OEL, N. NOEL, G. F. HAGERMAN, M. G. DUVAL
Univ. of Alberta, Edmonton, AB, Canada

Abstract: Our overarching Aim is to define signals required to regenerate cone photoreceptors, as this is a major hurdle for clinical stem cell therapy in restoring high-acuity, daytime and colour vision. We argue that rodent models are a poor model of cone regeneration because they are nocturnal and possess few cone photoreceptors in low density. Thus we turn to zebrafish with diurnal habit, ample cone photoreceptor density and excellent colour vision. Further, zebrafish undertake robust innate photoreceptor regeneration from intrinsic retinal stem cells. Here we assess photoreceptor and visual responses following conditional ablation of blue cone photoreceptors. Our previous work engineered zebrafish to show that ablation of UV cones biased regenerating cells to be cones (not rods) and especially UV cones (Fraser et al 2013, PMID: 23383182). We also nucleated understanding of a transcriptional network specifying blue and UV cones, involving *tbx2b* and the bmp ligand *gdf6a* (DuVal et al 2014, PMID: 24681822). We now observe results of ablating blue cones in zebrafish engineered with conditional ablation nitroreductase transgenes. We find that our novel constructs allow post-larval expression of our transgenes, overcoming a previous hurdle, as monitored with our novel fundus lens imaging technology (DuVal et al 2013, PMID: 23734077). We annotate effective ablation of blue cone photoreceptors upon application of the prodrug metronidazole. Specific visually-mediated behaviours were identified that are lost upon blue cone ablation. Intriguingly, these behaviours reappeared within days of cone ablation, implicating regeneration, continued retinal growth and/or synaptic plasticity as mechanisms of restoring visual function. We conclude that (i) our novel models of cone photoreceptor ablation will now permit ablation of blue cone photoreceptors, enabling comparison to regenerative events following UV cone ablation, thus addressing several hypothetical requisite cellular and signaling events; (ii) our latest generation

constructs allow ablation of specific cone classes later in development than was previously practical, including study of cone-subtype-specific regeneration in adults; (iii) integrating these models with the burgeoning knowledge of photoreceptor specification pathways will inspire clinical development of stem cell therapies to repair loss of daytime vision.

Disclosures: W.T. Allison: None. **A.P. Oel:** None. **N. Noel:** None. **G.F. Hagerman:** None. **M.G. DuVal:** None.

Poster

433. Photoreceptors

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Topic: D.04. Vision

Support: Award number VFGK048345

Title: An shRNA-based *in vivo* retina screen identifies molecules that regulate presynaptic development of rod photoreceptor

Authors: *S. KIM[1], C. PARK[2], T. COGLIATI[2], M. BROOKS[2], R. FARISS[2], W. LI[3], A. SWAROOP[2]
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Abstract: Our brain has a morphological variety of endless types of neurons. Answers about how such diversiform neurons develop and how specific circuits form among specific groups of neurons are still veiled. Photoreceptor in retina is a highly specialized neuron type to detect photons and transduce visual signals into second-order neurons, and finally transmit visual information into brain. The presynapses of rod and cone photoreceptor called spherule and pedicle respectively have specialized ribbon structure, which are different from conventional presynapses in brain. Further spherule and pedicle exhibit differential morphologies and connections with distinct sets of bipolar neurons in the distinct sublaminae of retinal outer plexiform layer (OPL). However, the molecular mechanisms that generate distinct ribbon synapses between spherule and pedicle remain unknown. Here, we wanted to understand the molecular mechanisms by which presynaptic morphogenesis and OPL sublamination of rod photoreceptor, and we report that transcription factor NRL and the downstream effectors are critical for the rod photoreceptor presynaptic morphogenesis We found that Nrl-/- rod photoreceptor presynapses (spherules) display cone photoreceptor presynaptic pedicle-like

forms, as Nrl-/- photoreceptor itself is differentiated via defaulted S cone pathway, and exhibit the presynaptic terminals in the lower portion of retinal OPL under rod bipolar dendritic tips, like wild type (WT) pedicles. To identify candidate genes that regulate the presynaptic morphogenesis of rod photoreceptor, the gene ontology analysis of Nrl downstream targets (Chromatin immunoprecipitation-Seq) which are enriched in WT retina (RNA sequencing between Nrl-/- and WT retinas) was performed, and shRNAs were used to decrease the expression of candidate genes in mouse retina especially, rod photoreceptors using *in vivo* retina electroporation. We found several shRNAs of Nrl down targets to disturb synaptic areas of rod photoreceptors. Our data indicate that one of fate determinate TF and its targets control the synaptic development of specific neuron-type, suggesting that fate determinate TFs are responsible for multiplex neuronal synaptic morphogenesis and their connections via a variety but specificity of synaptic molecular composition in different types of neurons.

Disclosures: S. Kim: None. **C. Park:** None. **T. Cogliati:** None. **M. Brooks:** None. **R. Fariss:** None. **W. Li:** None. **A. Swaroop:** None.

Poster

433. Photoreceptors

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Topic: D.04. Vision

Support: National Basic Research Program of China (973 Program, 2011CB707501)

National Natural Science Foundation of China (81100669)

Natural Science Foundation of Guangdong Province, China (S2012010008874, Y.X)

Title: The electroretinogram of Mongolian gerbil (meriones unguiculatus)

Authors: S. YANG[1], G. XIONG[1], X. LUO[1], K.-F. SO[1], J. DONG[2], *Y. XU[3,1]
[1]GHM Inst. of CNS Regeneration, [2]Dept. of Pathophysiology, Med. Sch., Jinan Univ., Guangzhou, China; [3]Joint lab for Brain Function and Hlth., Jinan Univ. Med. Sch., Guangdong, China

Abstract: Mongolian gerbil (Meriones unguiculatus) is a diurnal rodent with 13% of photoreceptors are cones , thus has been suggested to complement rats and mice for studying retinal cone function and pathologies. Yet there is little description of electroretinogram (ERG)

recording of gerbils using the protocol widely applied on human and other animals. In this study, we tested gerbil with flashes at increasing intensities under both dark and light adaptations. Similar to human, the intensity-profile for scotopic b-wave amplitude fit well the Naka-Rushton equation. But no clear “photopic hill” was observed in gerbil as in human and two other cone-rich rodents, Nile grass rat (35% of cones) and guinea pig (8-17% cones). We further checked the effect of repetitive application of chloral hydrate, the anesthesia used in clinical to sedate infants or children for ERG recording. The second ERG responses recorded 12, 24, 48 or 72 hours later were compared with the first recording in gerbil. Chloral hydrate significantly decreased scotopic a-wave, b-wave amplitudes and oscillatory potentials 48 hour after the injection. But there was little impact on photopic a-wave, b-wave, flicker responses and photopic negative response. Thus our results indicated a selective impairment of chloral hydrate on rod system, but not on ganglion cells and cones. As similar effects were also observed on children, we believe gerbil provides a valuable animal model to explore the possible underlying mechanism of the impact of chloral hydrate, and that ERG recording on gerbil has great potential to evaluate preclinical drugs or treatments on retinal neuron protection.

Disclosures: S. Yang: None. **G. Xiong:** None. **X. Luo:** None. **K. So:** None. **J. Dong:** None. **Y. Xu:** None.

Poster
(Unable to Attend)

433. Photoreceptors

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Topic: D.04. Vision

Support: UBACYT 2011-2014, 20020100100329.

CONICET PIP 1098.

Title: Adenosine A1 receptor changes throughout light induced retinal degeneration

Authors: *J. J. LOPEZ-COSTA, M. SOLIÑO, E. M. LÓPEZ, M. VACOTTO, E. GIRARDI
IBCN ¨Prof. E. De Robertis¨, F. Medicina, IBCN, Univ. De Buenos Aires-Conicet, Buenos Aires, Argentina

Abstract: Continuous illumination (CI) of rat retina produces photoreceptor degeneration. This model of light induced retinal degeneration (LIRD) resembles many of the characteristics of human retinal degenerative diseases such as Age-related Macular Degeneration (AMD), the first cause of acquired blindness in the developed countries, and Retinitis Pigmentosa (RP). The presence of A1 adenosine receptor has been reported mainly in ganglion cell layer of the retina by *in situ* hybridizaton, autoradiography and immunocytochemistry (ICC). In addition, A1 receptor agonists have been reported to be neuroprotective in animal models of inflammatory, hypoxic and degenerative diseases of CNS and retina. In order to shed some light on the processes underlying retinal degenerations and to asses a new potential therapeutic target, we studied the changes of A1 receptor in the retina in the model of LIRD by using ICC and Western Blot (WB) techniques. Sprague Dawley rats were submitted to CI (12000 lux) during 1, 2, 5 and 7 days. The eyes of the animals were removed and processed either by ICC or by WB. Both techniques were performed using an A1 receptor primary antibody (Santa Cruz). ICC and WB results were quantified by image analysis using the software Image J and Image Light Studio respectively. ICC data were statistically analysed using a one way ANOVA test. Immunocytochemical results showed immunoreactivity in nerve fiber layer, ganglion cell layer, inner plexiform layer, the inner portion of the inner nuclear layer and photoreceptor cell layer. Quantification showed an increase of the reactivity in all layers after 24 hs of CI. This first increase of optical density (OD) at day 1 was maintained steady during days 2 and 5 of CI. A second higher increase of OD was found at day 7. A one way ANOVA test of the results showed that all the observed OD increments throughout all retinal layers at days 1 to 7 were significant compared with controls ($P<0,0001$). The WB studies also showed an increase of A1 receptor levels compared to control levels from days 1 to 7 when the maximum relative density was also obtained. Our results showed an increase of A1 receptors in the retina starting at 24 hs which is the moment of maximal oxidative stress in coincidence with the peak of nitric oxide (NO) production. The observed increase of A1 receptor immunoreactivity may be the demonstration of an upregulation of adenosine receptors probably due to the lack of adenosine production during the retinal degeneration process. Knowing the beneficial effects of A1 receptor agonist in animal models of CNS diseases, A1R seems a likely target for future therapies of retinal degeneration.

Disclosures: J.J. Lopez-Costa: None. **M. Soliño:** None. **E.M. López:** None. **M. Vacotto:** None. **E. Girardi:** None.

Poster

433. Photoreceptors

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Title: *In vivo* evaluation of a novel small molecule antagonist of melanopsin

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Abstract: In the mammalian retina, the photopigment melanopsin is fundamental for non-image forming vision including light sensitivity, pupil constriction, sleep and alertness. Melanopsin uses a transduction mechanism in intrinsically-photosensitive retinal ganglion cells (ipRGCs) distinct from the rod/cone opsin signaling pathway. However, pharmacological agents that specifically target melanopsin signaling are unknown. Here we describe the identification of melanopsin-specific "opsinamide" antagonists. We used a high-throughput cell-based melanopsin photosensitivity assay to identify novel compounds from the sulfonamide family. *In vivo* administration of opsinamides to mice specifically and reversibly altered melanopsin-dependent light responses including photophobia and pupillary reflex to light (PLR) while leaving classical rod/cone photoreception largely unaffected (ERG). These results demonstrate that opsinamides specifically inhibit melanopsin signaling and modulate ipRGCs-dependent behaviors. The discovery of opsinamides raises the prospect of therapeutic control of the melanopsin system to regulate light-dependent behavior and remediate pathological conditions.

Disclosures: L.S. Mure: None. **K. Jones:** A. Employment/Salary (full or part-time):; Lundbeck Research USA Inc. **M. Hatori:** None. **S. Panda:** None. **J. Sprouse:** A. Employment/Salary (full or part-time):; Cyanaptic LLC.

Poster

433. Photoreceptors

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DFG Ha 5323/5-1

NIH EY021642

NIH EY014375

NIH EY007043

NIH EY023591

Title: Cone-by-cone threshold variability in the human retina

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Abstract: Recent studies have shown that cone photoreceptor weighting onto single retinal ganglion cells varies *in vitro* (Field et al. 2010; Li et al. 2014). With the advent of cone-specific microstimulation in the living eye (Yang et al. 2010; Harmening et al. 2014), we wondered whether this variation in cone weighting could also be observed at the perceptual level. Microstimulation enables the investigator to target light delivery to single cone photoreceptors repeatedly and reliably in the human eye, and do so day after day. By measuring increment thresholds of the same cones over multiple days, we sought to establish (1) whether perceptual thresholds arising from single cones are consistent over time, and (2) if the thresholds of adjacent cones vary. To accomplish this, cones were imaged using a multiwavelength adaptive optics scanning laser ophthalmoscope (AOSLO) with infrared light (842±25 nm), while tracking eye motion over a 1.2° patch of retina. We selected adjacent cones within the stabilized cone mosaic for targeted delivery of a 45 arcsec (~3.6 μm) square of 543 nm light, flashed for 130 μs. Stimuli were delivered after measurement and correction of chromatic aberrations (Harmening et al. 2012). Subjects (n=6) had normal color vision, and cones were located 2-3.5° from the fovea. Cones were tested in groups of 2 or 3, and each set of cones was tested 3-5 times within a single experiment. Thresholds were measured using a self-paced, 20-trial Bayesian staircase method

(ZEST). Staircases were pseudo-randomly interleaved for stimulation of each single cone individually. In 22 of 46 cone pairs, we found thresholds to differ significantly between cones. To assess the consistency of this threshold variability, 6 additional groups of cones were repeat tested over 2 or 3 days. In 4 of these cases, relative cone thresholds did not differ within the group, and persisted over time. In the other 2 cases, cones consistently exhibited significant differences in threshold. Our results suggest that individual cones may contribute differentially to downstream neurons, and that this can be detected at the psychophysical level. Additionally, our results indicate that increment thresholds of single cones are stable over short time periods.

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Poster

434. Striate Cortex Input Circuits

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Support: The study was performed as part of the EU-AIMS project that is funded by the Innovative Medicines Initiative.

Title: The Yin and Yang of perceptual grouping: Effects of glutamatergic and GABAergic compounds

Authors: *J. C. TALPOS, III[1], J. RIORDAN[1], J. OLLEY[1], J. WADDELL[1,2], T. STECKLER[1]
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Abstract: Perceptual grouping (PG), based upon spatial proximity, is disrupted in many cognitive disorders, including schizophrenia, Alzheimer's disease, and potentially autism. Yet few tools exist to study visual perception in a pre-clinical setting. However, rats can complete a visual discrimination using stimuli that differ only in their spatial proximity, which may serve as a test of PG. PG is thought to be dependent upon area V1 (primate) and is believed to be subject to the relative balance of glutamatergic excitation and GABAaergic inhibition of local cell networks. Evidence suggests that PG is disrupted by the excitation caused by NMDA antagonists. However, the effects of increased inhibition on PG remain unexplored. Accordingly, we tested the effects of the NMDA antagonists MK-801 and ketamine, and the GABAergic

allosteric modulator chlordiazepoxide (CDP) on performance of PG in rats. 24 male Lister hooded rats (Harlan, Netherlands; 180-200g) were used for this study. Water was provided ad libitum, but food was restricted; animals were maintained at approximately 90% of free feeding weight levels. Standard 5-panel Med Associates operant chambers were used for testing, where one wall was removed and replaced with a touch-sensitive computer monitor (running K-Limbic software V 1.2; Conclusive Solutions). Rats were presented with two stimuli (horizontal or vertical dots with a 2.75 spacing ratio) and were required to learn that a response at one stimulus would result in a food pellet reward, whereas a response at the other would not. During training, the image associated with reward remained the same for each rat. Sessions lasted for a total of 96 trials, or 45mins, whichever occurred first. Once stable performance had been achieved the distance between the dots was systematically altered (12 levels). The influence of ketamine (2.5-20 mg/kg), MK-801 (0.25-0.75 mg/kg) and CDP (1-10 mg/kg) was then evaluated (within subject design). MK-801 decreased accuracy across conditions, whereas ketamine interacted with difficulty, suggesting that it specifically impaired PG. Despite its sedating properties, the benzodiazepine CDP interacted with difficulty to improve PG. These data support the hypothesis that excitation induced by NMDA antagonists can disrupt PG (ketamine) and replicate previous results showing that they can impair performance of a visual discrimination (MK-801). Furthermore, we have demonstrated that increasing inhibition via activation of the GABAergic system may enhance visuo-perceptual abilities. These findings should increase our understanding of basic perceptual processes and of the etiology of perceptual impairments in CNS disorders.

Disclosures: **J.C. Talpos:** A. Employment/Salary (full or part-time):; Janssen Research and Development. **J. Riordan:** A. Employment/Salary (full or part-time):; Janssen Research & Development. **J. Olley:** A. Employment/Salary (full or part-time):; Janssen Research & Development. **J. Waddell:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutica NV. **T. Steckler:** A. Employment/Salary (full or part-time):; Janssen Research and Development.

Poster

434. Striate Cortex Input Circuits

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Program#/Poster#: 434.02/FF14

Topic: D.04. Vision

Title: Mixed excitatory/inhibitory interactions between V1 simple cells could reflect a Bayesian edge probability calculation

Authors: *G. C. MEL[1], C. A. RAMACHANDRA[2], B. W. MEL[1]
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Abstract: A key computation in visual cortex is the extraction of object contours, where the first stage of processing - local edge detection - is commonly attributed to orientation-tuned V1 simple cells. It is clear that compared to any one cell, far more information about the existence of an edge is encoded by a local population of cells with overlapping oriented receptive fields. It is not clear, however, how such a population should be decoded to calculate edge probability at a particular location, nor what type of neural circuit is capable of doing so. To gain insight into this population decoding problem, we collected filter statistics from neighborhoods in natural images when an edge was present, or not, at a central "reference" location. We then examined the form of the log-likelihood (LL) ratios for surrounding filters, which, within a Bayesian formulation, capture whether the neighboring filter should contribute excitation or inhibition to the reference edge probability calculation, or both, at different times. We found the LL ratios came in three main types: (1) essentially pure lateral inhibition, (2) monotonically progressing from excitation to inhibition as the neighboring filter value increases, and (3) non-monotonic, showing an initial increase in lateral excitation with increasing neighbor filter value, eventually waning and turning to inhibition as the filter value increases further. We constructed a simple model of the local cortical circuit that is capable of producing these types of lateral interactions through a combination of direct monosynaptic excitation and disynaptic inhibition. We conjecture that the mixed excitatory-inhibitory interactions frequently reported between nearby cells in V1 could reflect an underlying Bayesian edge probability calculation.

Disclosures: G.C. Mel: None. **C.A. Ramachandra:** None. **B.W. Mel:** None.

Poster

434. Striate Cortex Input Circuits

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Spanish Ministry of Education, Science (EX2009-0636)and Innovation

Title: Quantification and anatomical characterization of VGluT2-ir terminals in layer 4C of macaque primary visual cortex

Authors: *V. GARCIA-MARIN, T. H. AHMED, M. J. HAWKEN
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Abstract: In sensory cortex the excitatory drive in layer 4 arrives from two major sources: from thalamocortical (TC) and from intracortical (IC) afferents. The relative number and size of synapses from each source are important factors in establishing the efficacy of the different input sources. The goal of the current study was to estimate the proportion, size and organization of LGN (Lateral Geniculate Nucleus) synapses in layer 4C, the principal recipient layer of LGN afferents in primate V1. In earlier studies a variety of methods were used to estimate the proportion of synapses contributed by LGN afferents in the two sublayers of 4C, 4Cα and 4Cβ, such as: tracing studies, degeneration techniques, or immunohistochemistry. These studies have provided a wide range of estimates of the TC contribution to layer 4C, from 1.3% to 32% depending on species, sublayer, and microzones, with a median of around 8%. Recent studies indicate that TC terminals in V1 are primarily associated with the Vesicular Glutamate Transporter 2 – VGluT2 – which distinguishes them from IC glutamatergic axon terminals that express VGluT1. We have used confocal and transmission electron microscopic (TEM) techniques to quantify the number of VGluT2-ir terminals and analyze their ultrastructural properties in the different compartments of layer 4C. Both with confocal and electron microscopy we found the highest density of VGluT2-ir terminals in layer 4Cβ, accounting for around 16% of the total synapses. The density in layer 4Cα was 8% of the total. Using TEM we found that VGluT2-ir terminals in 4Cα were slightly larger (1.2±0.2 μm^2) than the terminals in 4Cβ (0.9±0.2 μm^2); however, both populations were 3-5 times much larger than non-VGluT2-ir terminals. The postsynaptic density (PSD) length was 1.2 times larger in the VGluT2-ir terminals than in the non-ir terminals. Additionally the VGluT2-ir synapses in 4C tended to establish multisynaptic input, whereas the non-ir synapses established one synapse per terminal. In layer 4Cβ VGluT2-ir terminals were preferentially associated with spines compared with non-VGluT2-ir terminals, whereas in 4Cα, both VGluT2- and non-VGluT2-ir terminals had a similar frequency of contacting with spines. The VGlut2 data is at the higher end of the range of the previous results, with the TC terminals providing 13% of the total synapses in layer 4C. The results also suggest that the efficacy of TC synapses, judged structurally, may be multifaceted. Taking into account that the TC synapses are much larger and establish more contacts per synapse with a larger PSD will be important in determining the overall contribution of TC input to the stellate cells in V1.

Disclosures: V. Garcia-Marin: None. **T.H. Ahmed:** None. **M.J. Hawken:** None.

Poster

434. Striate Cortex Input Circuits

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Title: Thalamocortical activation of Layer 4 neurons in primary visual cortex

Authors: *M. KLOC[1,2], A. MAFFEI[1,2,3]
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Abstract: The lateral geniculate nucleus (LGN) of the thalamus relays visual information from the retina to the primary visual cortex (V1), where it activates a complex layered circuit of excitatory and inhibitory neurons. Layer 4 (L4) receives the largest projection from the LGN. Although anatomical evidence suggests that thalamocortical (TC) inputs from the LGN innervate both excitatory and inhibitory neurons in L4 of V1 (Ahmed et al, 1997; Erisir et al, 2005), this input has yet to be functionally characterized. Here, we used an optogenetic approach to selectively activate TC afferents in acute coronal slices containing V1, and recorded postsynaptic currents from excitatory and inhibitory neurons using whole cell patch clamp (Wang et al, 2013). We show that TC inputs directly activate both excitatory neurons and one population of inhibitory neurons, the fast spiking (FS) cells; and that these inputs have cell-specific properties. TC-EPSCs recorded from excitatory and FS neurons differed in synaptic strength (Pyr=160±39.3 pA, N=29; FS=636.4±132.7, N=15; p=0.001) and suprathreshold activation (Spike/Stimulus: Pyr=0.8±0.07, N=22; FS=1.7±0.2,N=7; p=0.01). Thus, TC inputs drive FS neurons more strongly than excitatory neurons. Non-stationary noise analysis showed that TC-EPSCs recorded form excitatory neurons are mediated by a large number of open channels passing small unitary current. Differently, TC-EPSCs onto FS neurons depend on fewer open channels, with high unitary current (Pyr, n=211.2±50, i=4.3±1.1 pA, N=11; FS, n=22.7±3.6, i=26.2±6.1 pA, N=6; p=0.01). In addition TC-EPSCs onto pyramidal neurons comprised both AMPA and NMDA currents (AMPA:NMDA=0.27±0.06, N=10); while TC-EPSCs onto FS were mediated by an inwardly rectifying AMPA current (AMPA=554.6±90.7pA, N=5). In addition, TC-EPSCs had distinct short term dynamics onto pyramidal and FS neurons (PPR: Pyr=0.64±0.03, N=29; FS=0.43±0.07, N=15; p=0.05) suggesting that these input may have distinct presynaptic properties. To identify possible cell-type specific presynaptic mechanisms, we pharmacologically dissociated presynaptic release using 10mM strontium. TC-EPSCs onto FS

neuron decreased significantly more than those onto excitatory neurons, suggesting that TC inputs onto these two cell types are organized differently (ΔAmplitude: Pyr = 76.2±29.8 pA, N=17; FS = 170.8±27.1 pA, N=6; p = 0.03). Taken together, these data indicate that TC input onto L4 have cell-specific properties, which depend on a combination of pre- and postsynaptic mechanisms.

Disclosures: **M. Kloc:** None. **A. Maffei:** None.

Poster

434. Striate Cortex Input Circuits

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Support: Marie Cure CIG PCIG12-GA-2012-334353

Fundação para a Ciência e a Tecnologia SFRH / BPD / 88309 / 2012

Title: Interconnected layer 4 neurons in the mouse visual cortex receive common inputs from the lateral geniculate nucleus

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Abstract: The cerebral cortex is composed of intermingled neuronal networks. Cortical neurons receive inputs from neurons located in the same column as well as from long-range afferents projecting from other cortical areas and the thalamus. Sub-networks of locally interconnected neurons within the same layer share common translaminar local inputs and have similar functional properties *in vivo*. By differentially sampling local inputs, these local sub-networks are thought to constitute functional processing units. Ascending thalamocortical input relays sensory information to primary sensory cortices. In the primary visual cortex (V1), input from the dorsal lateral geniculate nucleus (dLGN) preferentially innervates neurons in layer(L)4. Recent studies have shown that L4 circuits boost thalamocortical visual information while preserving its tuning properties. Sampling of common dLGN input by recurrently interconnected L4 neurons could result in the observed amplification of thalamic signals. However, how locally interconnected neurons process long-range inputs remains unknown. Here, we studied how interconnected L4 neurons in V1 integrate dLGN inputs. By combining multiple whole-cell

patch-clamp recordings with Channelrhodopsin-2 (ChR2)-assisted circuit mapping, we developed a novel method to unravel how local and long-range connectivity relate to each other. ChR2 was expressed in dLGN neurons by stereotactic injection of adeno-associated viruses. Several days later, we prepared acute brain slices from ipsilateral V1. We simultaneously recorded from two or more L4 neighboring excitatory neurons while photostimulating afferent thalamocortical axons. Using a laser beam that could be rapidly repositioned with galvonemeter mirrors and low laser powers, we repeatedly minimally photostimulated one (or a few) ChR2+ axons in 64 different locations surrounding the recorded neurons. By analyzing the correlation of unitary (or oligoaxonal) photostimulation-evoked post-synaptic responses across simultaneously-recorded L4 cells, we quantified the amount of shared thalamocortical inputs for connected and non-connected cell pairs. We found that interconnected L4 neurons share 2.5-fold more common input from dLGN than non-connected neurons. Furthermore, the probability of receiving common dLGN input increased with the strength of the L4-L4 connections. Our results provide a circuit mechanism for the observed amplification of dLGN inputs by L4 circuits. These findings also validate a new circuit mapping method as a proper tool to study the interplay between long-range projections and local neuronal sub-networks.

Disclosures: **N.A. Morgenstern:** None. **L. Petreanu:** None.

Poster

434. Striate Cortex Input Circuits

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Title: The development of parvalbumin positive neurons in ferret visual cortex

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Abstract: The onset of visual experience is accompanied by dramatic changes in the response properties of neurons in the visual cortex. In the ferret, these changes include alterations in the magnitude and correlation of visually driven responses, and the emergence of selectivity for direction of stimulus motion. To explore the developmental changes in cortical circuitry that

might underlie these functional changes, we examined the maturation of parvalbumin-containing (PV) neurons, a subtype of GABAergic neurons that is the source of synapses onto neuronal cell bodies. Before eye opening, the pattern of parvalbumin immunoreactive cell bodies and terminals is quite different from that seen in the mature cortex. PV+ cell bodies and processes are prominent in layer 5, with fewer cell bodies and processes in layer 2/3; Notably, layer 4 exhibits few PV+ cell bodies and soma-targeting terminals. Within 3 days of the onset of visual experience, the distribution of PV+ cell bodies and terminals changes dramatically: PV+ cell bodies, processes, and soma-targeting synapses are present in layer 4 and throughout layers 2-6. To assess the role of visual experience in the developmental changes of PV neuron morphology, we compared the patterns of immunoreactivity in normally reared animals with the patterns in animals dark reared from P15 to P40. Compared with control animals of the same age, dark reared animals displayed a paucity of immunoreactive processes in layer 4, suggesting that visual experience plays a large role in the establishment of the mature pattern of PV immunoreactivity. To explore the potential functional implications of these developmental changes, we performed whole cell patch recordings on stellate cells - the targets of PV+ neurons - within layer 4. Consistent with the developmental emergence of PV+ soma-targeting synapses, we found a developmental increase in the frequency of mIPSCs in stellate cells. However, an increase in mIPSC frequency did not occur in time matched dark reared animals, where development of PV+, soma-targeting synapses is delayed. Together, these results indicate that rapid changes in the organization of this subclass of GABAergic neurons may play a significant role in the functional changes that accompany the onset of visual experience.

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Poster

434. Striate Cortex Input Circuits

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FAPERN

Title: Cortical parcellation, neuropil reactivity and distribution of calcium-binding protein immunoreactive neurons in the visual cortex of the marmoset (Callithrix jacchus)

Authors: *M. A. FREIRE[1], P. F. CAVALCANTI[2], T. G. MENDES[2], M. B. SANTANA[2], J. G. FRANCA[3]
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Abstract: The marmoset (Callithrix jacchus) is a small New World monkey species endemic to Brazilian Northeast, possessing a lissencefalic that ensures a facilitated access to its various regions. Such characteristics elect this species as a suitable model for studies of the Central Nervous System (CNS). In the present study, we aimed to characterize the distribution and reactivity pattern of neurons of the visual cortex revealed by immunohistochemistry for calcium binding proteins (CBP) calbindin (CB), calretinin (CR) and parvalbumin (PV) throughout the different visual areas of this species. Three male adult marmosets were used. The animals were perfused with 0.9% warm heparinized saline and 4% cool paraformaldehyde. Their brains were removed from the skull, cryoprotected with 20% sucrosis in 0.1M phosphate buffer(PB), divided in three blocks and frozen. The posterior block, containing the visual cortex, was sectioned coronally at 50 μm in a cryostat. Alternate sections were stained with cresyl violet (Nissl method) and CO histochemistry for delineation of cortical layers and areal borders. Immunohistochemistry for CBP (CB, CR and PV) was performed in another series of adjacent sections. After mounting, histological slides were subjected to qualitative and quantitative inspection using Neurolucida system. All experimental procedures were strictly in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and Local Ethics Committee (license 08-2011, CEUA/AASDAP). Both Nissl staining and CO-reactivity revealed a clear delimitation of primary (V1), secondary (V2) and tertiary (V3) visual areas. The distribution of CBP-reactive neurons was heterogeneous across visual areas. In V1, PV-reactive (PV-r) cells were found in both supra (SG) and infragranular (IG) layers, whereas CB-r cells were mainly distributed across IG and CR-r cells were found in SG. In V2, PV-r and CB-r cells were mainly concentrated in IG layers, while CR-r cells were found predominantly in SG. In V3, CB-r cells were homogeneously distributed across both SG and IG while CR-r cells were found especially in SG and PV-r cells in IG. The distribution of CBP-reactive neurons in the visual cortex of marmoset follows the pattern described for some other species of non-human primates (J. Chem Neuroanat 16: 77, 1999), suggesting that the pattern of cortical organization is phylogenetically conserved in this group. Both the pattern of distribution and reactivity along V1, V2 and V3 areas of the marmoset may be related to compartmentalization of information processing that occurs in different circuits of these cortical regions.

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Poster

434. Striate Cortex Input Circuits

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Title: A critical role of NMDA receptors in parvalbumin interneurons for visual information processing in mouse V1

Authors: *M. FIORINI[1], A. VAICELIUNAITE[1,2], S. ERISKEN[1], O. JURJUT[1], S. KATZNER[1], L. BUSSE[1]
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Abstract: In cortex, fast-spiking parvalbumin positive (PV+) interneurons represent the largest neuronal population mediating GABAergic inhibition. In primary visual cortex (V1), PV+ interneurons modulate the tuning properties of excitatory cells by contributing to selectivity for stimulus orientation, contrast and size. Like pyramidal cells, PV+ interneurons receive excitatory input through glutamate receptors, the disruption of which has been implicated in impairments of visual feature integration and figure-ground segregation (Self et al., 2012). Here we examined the impact of NMDA-glutamate receptor ablation in PV+ interneurons on visual information processing. We generated mice lacking NMDA receptors in PV+ neurons by crossing PV-Cre mice with mice carrying floxed NR1 alleles (NR1-PVCre$^{-/-}$; Korotkova et al., 2010; Carlen et al., 2012). We recorded V1 extracellular activity of head-fixed, transgenic and littermate control mice placed on a Styrofoam ball, where they could run or remain stationary. We assessed spatial integration by measuring size tuning curves with gratings of different diameters. We measured selectivity for orientation and contrast by presenting full-field gratings of different orientations and contrasts. We first compared overall neural activity, and found that both baseline and peak firing rates were largely similar between genotypes. We next tested the role of NMDA receptors in PV+ interneurons for spatial integration. Compared to control mice, V1 cells in NR1-PVCre$^{-/-}$ mice had smaller receptive field center sizes and stronger surround suppression. This difference in spatial integration between mutant and wildtype mice persisted even after equating for differences in their locomotion behavior, such as time spent running and average running speed.

Since previous studies suggested that PV+ interneurons might shape spatial integration via contrast gain control mechanisms (Vaiceliunaite et al., 2013; Nienborg et al., 2013), we also investigated whether NMDA receptors in PV+ interneurons contributed to contrast sensitivity. Compared to control mice, V1 cells in NR1-PVCre$^{-/-}$ had higher sensitivity for stimulus contrast (i.e. lower semisaturation contrasts c_{50}). We speculate that this higher sensitivity could, at least partly, mediate sharper tuning for stimulus size. We conclude that NMDA transmission in PV+ interneurons shapes selectivity of neighboring pyramidal cells for stimulus size, possibly via mechanisms of contrast gain control.

Disclosures: **M. Fiorini:** None. **A. Vaiceliunaite:** None. **S. Erisken:** None. **O. Jurjut:** None. **S. Katzner:** None. **L. Busse:** None.

Poster

434. Striate Cortex Input Circuits

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Title: Circuit mapping of basal forebrain inputs to inhibitory interneurons in the neocortex

Authors: ***M. FOER**[1], M. E. RECH[1], C. R. CADWELL[3], J. REIMER[3], A. S. TOLIAS[3,2]
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Abstract: The basal forebrain (BF), which is comprised of several nuclei located rostrally and ventrally to the striatum, is a major source of cholinergic input to the neocortex and is thought to play an important role in modulating cortical networks during cognitive processes such as attention and learning. For example, electrical stimulation of the BF improves sensory encoding of visual stimuli by reducing correlated variability and increasing the reliability of neural responses in primary visual cortex (V1). However, the mechanisms by which the BF modulates cortical networks remain poorly understood. In addition to cholinergic projection neurons, the BF also contains GABAergic and glutamatergic projection neurons and it has been suggested

that cholinergic and non-cholinergic BF projections may have unique roles in shaping cortical responses. Here, we map the targets of cholinergic and non-cholinergic BF projections to different neuronal subtypes in V1. We utilize a transgenic approach to selectively target parvalbumin (PV)-, somatostatin (SST)-, or vasoactive intestinal peptide (VIP)-expressing interneurons in V1 for retrograde rabies virus tracing and quantify the number of cholinergic and non-cholinergic inputs that each cell type receives from different BF nuclei. Interestingly, we find that different cell types in the neocortex receive inputs selectively from distinct populations of neurons within the BF. Our findings suggest that BF activation may differentially modulate interneuron subtypes to enhance information processing in the neocortex.

Disclosures: **M. Foer:** None. **C.R. Cadwell:** None. **J. Reimer:** None. **A.S. Tolias:** None. **M.E. Rech:** None.

Poster

434. Striate Cortex Input Circuits

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Topic: D.04. Vision

Title: Morphological analysis of the layer 6 input to layer 4 in primary visual cortex of the mouse

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Abstract: Corticothalamic neurons form major excitatory projections to the thalamus and to cortical layer 4. In mouse visual cortex, layer 6 activation mediates suppression of neurons in upper layers. This is a remarkable finding since in rat and cat, layer 6 corticothalamic cells target mostly excitatory neurons. In order to investigate the mechanisms of layer 6 action, we reconstructed from serial EM sections the synaptic boutons of layer 6 cells and identified their synaptic targets in mouse primary visual cortex. We used the mouse line Ntsr1-Cre with Td tomato reporter that labels layer 6 neurons in V1 and then used an antibody against red fluorescent protein visualized by nickel intensified diamino benzidine (DABNi) to obtain an electron dense label in these neurons. Locations in layer 4 of V1 were then chosen using a systematic random sampling scheme and 43 synapses formed by layer 6 neurons were reconstructed in 3D at electron microscope level using 40 nm thick serial sections. The majority of targets of layer 6 boutons in layer 4 were spines (58%). Dendritic shafts were targeted in 30%

of cases and in 12% of the reconstructions we could not unambiguously identify the target. Spines targeted by layer 6 boutons were often small and of the stubby type. Synapse formed by layer 6 cells in layer 4 were also usually small with an average area of postsynaptic density (PSD) of 0.04 μm2. The average PSD density for spine targets was 0.04 μm2 (std = 0.02) and for shaft targets was 0.045 μm2 (std=0.016). There was no statistical difference between labeled synapses formed with spines and shafts (p = 0.045, t test). Our results show that in mouse V1 the layer 6 projection to layer 4 targets predominantly spiny (putative excitatory) neurons. This suggests that the suppression observed in layer 4 by the activation of layer 6 is not obtained by the specific targeting of inhibitory neuron in layer 4.

Disclosures: **A.L. Bodor:** None. **M. Takeno:** None. **N.M.D. Costa:** None.

Poster

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Title: Functional contribution of direct geniculocortical input to deep layers of visual cortex

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Abstract: We investigated the role of direct thalamocortical input to deep layers of visual cortex. Sensory information from the periphery is relayed to the cerebral cortex by thalamocortical axons, which terminate primarily in layer 4 (L4). The canonical model for cortical circuitry proposes that information is processed serially from L4 to L2/3, then to the infragranular layers, L5 and L6. However, thalamocortical axons also terminate to a lesser degree in the deep layers, and L5 pyramidal neurons in barrel cortex receive substantial direct thalamocortical input. Recent work demonstrated that pharmacological inactivation of layer 4 in the somatosensory

cortex has little or no effect on sensory-evoked synaptic input to L5 neurons within the same barrel column, revealing that primary thalamic nuclei may initiate cortical processing via two distinct layers (L4 and L5B). A major unresolved question is whether these findings are specific to barrel cortex or are true of all neocortical sensory systems. In the primary visual cortex (V1) of mouse, cat, monkey, and human, thalamocortical axons bifurcate in L5 as well as L4, similar to rodent S1. Here, we test whether thalamic axons can directly drive L5 neurons in mouse V1 independently of the LGN-L4-L2/3 pathway. Using whole-cell patch clamp recordings of L5 pyramidal neurons during visual stimulation, we show that L5 neurons retain robust sensory responses, and assess how these responses are influenced by inactivation of upper cortical layers.

Disclosures: A.K. Kinnischtzke: None. **Y. Hong:** None. **R.M. Bruno:** None.

Poster

434. Striate Cortex Input Circuits

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Title: Morphological comparison of inputs to primate visual areas MT, V1 and V2

Authors: R. T. MARION, K. LI, J. A. MAVITY-HUDSON, *V. A. CASAGRANDE
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Abstract: The flow of visual information beyond the primary visual cortex (V1) is of renewed interest as recent studies have emphasized the role of the thalamus, specifically the pulvinar as

the potential conduit of visual signals between visual cortical areas. Previously, we demonstrated that the lateral pulvinar (PL) projections to the second visual area (V2) ends in layer 4 and has bouton sizes comparable to parvocellular (P) layer projections from the lateral geniculate nucleus (LGN) to V1 layer 4, but smaller than LGN magnocellular (M) layer projections to V1 layer 4, and significantly larger than V1 projection boutons to V2 layer 4 (Marion et al., 2013). Those results suggested that PL could provide the major driving visual input to V2. The middle temporal visual area (MT), which has significant roles in motion detection and attention, receives major projections from V1 that have beaded and large boutons and that also end in layer 4, showing features of a driving input. Thus, MT can be considered the next visual processing stage after V1. At the same time MT receives from many other cortical and subcortical areas, including the pulvinar. In this study we compared the axonal distribution and bouton sizes of LGN inputs to V1 with pulvinar inputs to two extrastriate areas, MT and V2, in the prosimian bush baby. In the current study we first compared pulvinar to MT projections with other known projections in the early visual system. Briefly, we pressure injected 300nl biotinylated dextran amine (BDA 10000 MW) into the shared central vision representation of the two retinotopic maps in PL, after electrophysiologically confirming their locations with a single tungsten electrode glued to the pipette. After a survival period of 2-4 weeks, we perfused the animals and visualized the BDA labeled pulvinar terminals with fluorophore conjugated streptavidin. High-power confocal stacks were taken from the center of projection foci which were used to identify boutons by a naive observer. Each identified bouton's area was determined by its contour at half brightness compared to the local maximum. Bouton size comparisons were done only between injections matched based on pulvinar map retinotopy. PL axons show dense projections to layer 4 of MT. We found, however, that PL boutons in MT were significantly smaller than either PL boutons in V2 or LGN P cell boutons in V1. The PL to MT boutons were the same size as the V1 to V2 boutons (Marion et al., 2013). Since larger bouton size has been correlated with driving projections, this finding supports a modulatory role for the projections from pulvinar to MT, a result which would require specific functional tests to confirm.

Disclosures: R.T. Marion: None. **K. Li:** None. **J.A. Mavity-Hudson:** None. **V.A. Casagrande:** None.

Poster

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EY008126

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HD15052

Title: Mechanisms of pulvinar control of the primary visual cortex (V1)

Authors: *K. LI[1], G. PURUSHOTHAMAN[2], J. A. MAVITY-HUDSON[2], Y. JIANG[1], D. YAMPOLSKY[2], V. A. CASAGRANDE[2,3,4]
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Abstract: The lateral pulvinar (PL) forms reciprocal connections with V1 in primates. The projections from pulvinar to V1 end mostly in layer 1 where they are able to excite pyramidal neurons in layer 2/3 (Purushothaman et al., 2012), the output neurons to higher order cortical areas. In order for pulvinar input to excite V1 output cells this input must have a mechanism to overcome the heavy inhibition on V1 layer 2/3 pyramidal neurons. However, the synaptic targets of PL axons in V1 have never been examined before. V1 layer 1 primarily contains GABAergic interneurons including single bouquet cells (SBC) and neurogliaform cells (NGFC), but also the apical dendrites of pyramidal cells in layer 2/3 and layer 5. PL synapsing directly with pyramidal output cells could boost output signals directly. SBC synapses with other interneurons, and could disinhibit pyramidal cells. NGFC form wide spreading networks with gap junctions, and could mediate strong inhibitory effects on pyramidal cells. Metabotropic glutamate receptor 2 (mGluR2) is expressed heavily in V1 layer 1. mGluR2 is expressed on both pre- and post-synaptic membranes, where it could reduce postsynaptic excitation, and potentially meditate inhibition of target interneurons by glutamatergic pulvinar input. Therefore, there are a number of ways the PL input could control V1 output. As a first step to understand how PL input to V1 regulates V1 output cells we examined the synaptic arrangements made by labeled pulvinar-V1 axons at the electron microscopic level. Specifically, we examined whether pulvinar projections to V1 form direct connections with pyramidal cells or with interneurons. We used adult bush babies of both sexes in this study. Pulvinar terminals in V1 were labeled with biotinylated dextran amine injections made into the visual field representations of lateral and inferior pulvinar, and were visualized using preembedding streptavidin-HRP/tetramethylbenzidine with diaminobenzidine stabilization after a survival period of 3-11 weeks. Ultra-thin sections were double labeled with antibodies against mGluR2 and GABA, and visualized with 15nm and 25nm gold particle conjugated secondary antibodies. Preliminary results suggest that pulvinar axons synapse on spines of presumed pyramidal cell dendritic tufts and not on interneuron cell bodies or dendritic shafts. These early results support the idea that pulvinar axons in V1 could directly

activate V1 output cells. Since no clear pattern of mGluR2 label was found to date, it is still unclear how this receptor relates to the inhibitory circuitry in V1 and whether pulvinar mediated disinhibition additionally contributes to pulvinar's control of V1.

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Poster

434. Striate Cortex Input Circuits

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Title: Visual processing in V1 by fast and slow corticogeniculate neurons: axonal conduction time matters

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Abstract: Corticogeniculate (CG) neurons of layer 6 of primary visual cortex (V1) provide feedback projection to the lateral geniculate nucleus (LGN) that is thought to shape receptive field properties and influence spike timing and response gain of thalamic neurons. In both cats and rabbits, however, CG axons display an enormous range of corticothalamic conduction times (>40.0 ms, Ferster and Lindstrom, 1983; Swadlow and Weyand, 1987), and little is known how such variations are related to receptive field tuning and visual responding. In this study, we investigated the response properties of 54 antidromically identified CG neurons in the awake rabbit, and visual responses were characterized quantitatively using sparse noise and drifting gratings. For various tests, the size, orientation, spatial frequency, contrast, and temporal frequency of the stimuli were optimized for each neuron. Thirty-one of these cells responded to visual stimulation, had simple receptive fields and had antidromic latencies of 2.5 to 35 ms (mean = 18.8 ms). Although spontaneous activity levels were similarly low for both fast and

slow CG neurons (median = 0.15 spike/s), fast CG neurons differed in their visual response properties from those with slowly conducting axons in several ways. (1) The spatio-temporal receptive fields for fast CG neurons had shorter receptive field onset latency ($r=0.67$, $p<0.001$) and time to receptive field peak ($r=0.66$, $p<0.001$). (2) Fast CG neurons were more sensitive to contrast (C-50) than slow CG neurons ($r= 0.55$, $p<0.02$), and (3) preferred higher velocity stimuli ($r= -0.40$, $p<0.03$). (4) During optimal drifting grating stimulation fast CG neurons were more responsive (higher F1) than slow CG neurons ($r = -0.71$, $p < .001$) and (5) had shorter minimal interspike intervals ($r= .73$, $p<0.001$). Additionally we recorded from 23 CG neurons which were identified by antidromic activation, yet they showed no spontaneous firing and were not reliably activated by any visual stimuli. These "silent" cells had longer antidromic latencies (9.4 to 49 ms (mean = 30.6 ms, t-test $t = 4.21$, $p = 0.001$) than visually responsive CG neurons. Taken together these results indicate that the diversity in CG axonal delays is strongly related to the diversity in the visual response properties of this system.

Disclosures: Y.I. Bereshpolova: None. **C.R. Stoelzel:** None. **J. Zhuang:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

434. Striate Cortex Input Circuits

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KAKENHI 25117004, JSPS

Title: Functional imaging of thalamic axons in mouse primary visual cortex

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Abstract: The prominent aspect of primary visual cortex (V1) is the response selectivity to visual stimulus features. Layer 4, the major entrance layer of V1, receives visual input from lateral geniculate nucleus (LGN) of thalamus. Layer 4 excitatory neurons in mouse V1 possess high selectivity for stimulus orientation. Recent studies indicated that thalamocortical axons

targeting layer 1, another recipient layer of thalamic input, had sharp orientation selectivity (Cruz-Martin et al., 2014). However, it is still unknown whether thalamocortical axons arborizing in layer 4 also carry sharp orientation tuning. To answer this question, we performed *in vivo* two-photon calcium imaging of thalamocortical axons in mouse V1 and investigated their response selectivity to visual stimuli. For axonal calcium imaging, GCaMP6s, genetically encoded calcium indicator, was locally expressed in LGN neurons by AAV-mediated method. Boutons of thalamocrtical axon expressing GCaMP6s were clearly visible in layers 1 and 4 of primary visual cortex and we could record their response selectivity to visual stimuli. We found thalamocortical axons arborizing in layer 4 had broad orientation selectivity, while axons in layer 1 had mixture of sharp and broad orientation tuning. These results suggest that layer 4 neurons receive largely untuned input from LGN, although they have sharp orientation selectivity.

Disclosures: S. Kondo: None. **K. Ohki:** None.

Poster

434. Striate Cortex Input Circuits

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Title: Signatures of Magnocellular and Parvocellular inputs to cortical LFP

Authors: *M. JANSEN[1], X. LI[1], R. LASHGARI[1], J. KREMKOW[1], Y. BERESHPOLOVA[3], H. SWADLOW[3], Q. ZAIDI[2], J.-M. ALONSO[1]
[1]Biol. Sci., [2]Grad. Ctr. for Vision Res., SUNY Col. of Optometry, New York, NY; [3]Psychology, Univ. of Connecticut, Storrs, CT

Abstract: The primary visual cortex (area V1) receives input mainly from two thalamic pathways, Parvocellular and Magnocellular, that differ in chromatic selectivity and spatial resolution. Parvocellular neurons respond stronger to equiluminant chromatic gratings and are

tuned to higher achromatic spatial frequencies than Magnocellular neurons. Consistent with these differences, we found that local field potentials (LFPs) that are significantly modulated by equiluminant chromatic gratings (chromatic LFP) have higher achromatic spatial resolution than those that are not modulated (achromatic LFP). We measured cortical LFPs with an array of ultrathin electrodes that were independently movable and chronically implanted in area V1 of two awake macaques (Swadlow et al., 2005). The LFP spatial frequency tuning was measured with light-dark achromatic and equiluminant-red-green sine-wave gratings (.01 to 6 cpd). LFP sites were classified as chromatic based on two criteria: 1) the LFP response was significantly stronger to equiluminant red-green gratings than light-dark gratings; 2) the responses to equiluminant red-green gratings plus 10% contrast light-dark gratings were roughly invariant to the relative phase of the added grating. Chromatic and achromatic LFP sites differed in spatial resolution, low-spatial frequency suppression, peak-to-trough amplitude and their cortical depth. The spatial resolution measured with light-dark gratings was ~50% higher for chromatic than achromatic LFP sites, as estimated by the peak spatial frequency (3.1± 0.44 vs. 2.0 ± 0.25 cpd, p=0.037, t test) and spatial frequency cut off (5.1 ± 0.33 vs 3.4 ± 0.40 cpd, p = 0.001, t test). Chromatic LFPs were also more strongly suppressed by low spatial frequencies of light-dark gratings (ratio between maximum response and response to lowest spatial frequency tested: 1.6 ± 0.11 vs. 1.3 ± 0.05, p=0.021, t-test) and had more positive peak-to-trough amplitudes (with achromatic stimuli: 0.114 vs. -0.175, $p < 0.0001$, t-test; with chromatic stimuli: 0.108 vs. -0.175, $p < 0.0001$, t-test). Finally, the LFP polarity (measured within 60 msec of the stimulus onset) was near zero for chromatic LFPs (indicating a location near the input layers) and negative for non-chromatic LFPs (indicating a more superficial location, 0.07 vs. -2.96, p=0.047). In summary, our results indicate that the LFPs can resolve differences in achromatic spatial resolution that are likely to originate from Parvocellular and Magnocellular inputs to primary visual cortex. These differences could potentially be used to assess the function of Parvocellular and Magnocellular pathways in cortical implants.

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Poster

434. Striate Cortex Input Circuits

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Support: HHMI

Title: The lateral geniculate nucleus provides layers 1 through 4 of primary visual cortex with orientation- and direction-selective inputs

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Abstract: Primary visual cortex (V1) conveys orientation- and direction-selective visual information to the rest of the brain. Most models of how orientation selectivity arises assume that circuits within V1 compute these features from non-orientation-tuned inputs originating from the dorsal lateral geniculate nucleus (dLGN), which sends projection to both the supragranular layers (L1 and L2/3) and the granular layer (L4) of V1. However, almost nothing is known about orientation- and direction-tuning of dLGN inputs to V1, apart from a recent analysis of a small number of neurons suggesting that dLGN provides tuned inputs to layer 1, but not layer 4. Here, we characterize the orientation- and direction-tuning properties of the major inputs and outputs of mouse V1 by *in vivo* calcium imaging of ~28,000 dLGN boutons and 1,500 layer 5 neurons. Contradicting the prevailing view that the L4 geniculate inputs are not orientation-tuned, we find that around half of the dLGN inputs to L4 carry direction- or orientation-tuned information. The direction-selective inputs across all layers strongly prefer the posterior-to-anterior motion direction, while the orientation-selective inputs are dominated by the horizontal motion in all layers with a substantial component of the vertical motion in L1. The same posterior-to-anterior direction dominates the direction-selective L5 neurons, while a majority of the orientation-selective L5 neurons tune to either the horizontal or vertical orientation. Direct synaptic connections are found between the geniculate afferents and the L5 neurons, suggesting that the thalamic inputs may directly drive cortical outputs.

Disclosures: **W. Sun:** None. **N. Ji:** None.

Poster

435. Decision Making and the Cortex

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Title: Attention has opposite effects on spike count correlations within and between visual areas

Authors: *D. A. RUFF, D. F. MONTEZ, M. R. COHEN
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Abstract: Neurons in multiple visual cortical areas typically respond to many types of visual stimuli and carry information that might be useful for solving a variety of perceptual tasks. For example, neurons in both areas MT and V1 carry information that would be useful for solving a motion direction change detection task. It is unknown, however, whether information from multiple areas is combined without regard to which area the neurons are in or whether the visual system processes information in a more sequential manner (e.g. with neurons from downstream areas reading out information from earlier processing stages). We hypothesized that spatial attention, which improves perception at the attended location, would allow us to probe how visual information encoded in V1 and MT is combined to solve a direction change detection task. Previous studies have shown that shifting attention towards the joint receptive field of a pair of neurons within the same cortical area tends to decrease the extent to which the trial-to-trial fluctuations in their responses are correlated (termed spike count correlations). We reasoned that if information from V1 and MT is combined without regard to the cortical area of origin, then attention should decrease correlations between areas just as it does within areas. Conversely, if attention affects the extent to which MT responses reflect the activity of specific subgroups of V1 neurons, then attention should increase correlations between MT and the V1 neurons that encode the attended stimulus. We recorded simultaneously from a few dozen V1 neurons and a single neuron in MT with overlapping receptive fields in two monkeys. During the recordings, the monkeys performed a direction change detection task that required them to shift attention between two moving stimuli within the MT neuron's receptive field. We found that attention had opposite effects on correlations within and between cortical areas. Consistent with previous studies, we found that attention decreased correlations between V1 neurons with overlapping receptive fields. However, attention increased correlations between the MT neuron and the V1 neurons whose receptive fields overlapped the attended stimulus. Our study demonstrates that attention has qualitatively different effects on groups of neurons within and across cortical areas.

These results suggest that in addition to affecting the way sensory stimuli are encoded within a cortical area, attention may affect the transmission of sensory information between cortical areas.

Disclosures: D.A. Ruff: None. **D.F. Montez:** None. **M.R. Cohen:** None.

Poster

435. Decision Making and the Cortex

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Frieda Martha Kunze Fellowship

Title: Correlations within V4 populations do not affect sensory encoding or choice decoding in a rapid shape detection task

Authors: *K. F. WEINER[1], G. M. GHOSE[2]
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Abstract: Depending on how sensory information is encoded among neurons, and how these neurons are decoded, correlations could either help, hinder, or have no effect on sensory-based decision making. Many studies have approached this issue by measuring correlations between the sensory responses of neuron pairs over hundreds or even thousands of milliseconds. However, in most real world situations, sensory activity over large neuronal populations must be rapidly sampled to direct relevant behavior. It is therefore unclear how pairwise and higher order correlations over these smaller timescales affect the fidelity of stimulus encoding across populations or the physiological decoding of that population activity to initiate actions. To address these questions, we trained two male monkeys (Macaca mulatta) to rapidly detect a shape that randomly appeared within a noise stimulus. The animals were required to report the appearance of a shape by making a saccade within 550 ms of its appearance; average reaction times were ~250 ms for both animals. To investigate the neuronal basis of this rapid shape detection at a population level, we chronically implanted a microelectrode array in area V4, allowing for simultaneous recording of 10-75 neuronal units while the animals performed the

detection task. We used a linear discriminant analysis to quantify the mutual information between the recorded populations and the stimulus (no shape/shape) as well as between the population and the animals' detection behavior (fixation/saccade) on a moment-by-moment basis. All neuronal populations (from a total of 20 recording sessions) carried significant information about both the preceding stimulus and the subsequent eye movements over timescales relevant to behavior, suggesting a potential role in the detection decisions made by the animals. Neuronal populations were much more informative than single cells and typically performed approximately half as well as the animals themselves. To determine the effect of correlations on population performance, we shuffled observations within each stimulus (no shape/shape) and behavioral (fixation/saccade) category to remove both pairwise and higher order correlations in neuronal variability. For all populations, we found that these shuffled populations conveyed stimulus information just as well as the actual population. Additionally, shuffled and actual population responses were also equally capable of predicting the animal's behavior on a moment-by-moment basis. This suggests that in our rapid shape detection task, the correlations among area V4 neurons neither affect the fidelity of stimulus encoding nor impact the animal's behavior.

Disclosures: K.F. Weiner: None. **G.M. Ghose:** None.

Poster

435. Decision Making and the Cortex

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 435.03/FF32

Topic: D.04. Vision

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Gatsby Charitable Foundation

Title: Spatial attention may regulate noise correlations through increases in local inhibition

Authors: *J. F. MITCHELL, J. H. REYNOLDS
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Abstract: Recent studies in non-human primates have found that spatial attention reduces the variability of neuronal responses, thereby improving perception. Much of this variability originates from ongoing activity that is shared across populations (i.e., so-called "noise"

correlations). I will outline a series of experiments that illuminate the neural mechanisms underlying attention-dependent reductions in variability. First I show that spatial attention increases local inhibition, as indicated by larger gain increases among putative interneurons than pyramidal cells. Spatial attention also causes reductions in the noise correlations, and this effect is shared across both putative interneurons and pyramidal cells. I introduce a realistic spiking network model that accounts for these findings and illustrates a possible mechanism by which local inhibition could regulate noise correlations. In the model, noise correlations arise from spontaneous waves of activity that are spread through the population via recurrent connections to distal dendrites of pyramidal neurons. Increases in local inhibition act to shunt away the influence of this distal input, thereby reducing the impact of spontaneous waves and reducing noise correlations. The model makes a falsifiable prediction that inhibition should be increased by an indirect route mediated through excitatory feedback to deep layer pyramidal neurons. Rather than directly targeting interneurons, excitatory feedback should target deep layer pyramidal neurons that preferentially drive interneurons in superficial layers. If so, deep layer pyramidal neurons should exhibit larger gain increases with attention than superficial layer pyramidal neurons, but both populations should exhibit similar reductions in noise correlations.

Disclosures: J.F. Mitchell: None. **J.H. Reynolds:** None.

Poster

435. Decision Making and the Cortex

Location: Halls A-C

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Topic: D.04. Vision

Support: CIHR Grant MOP-115178

CIHR Award GSD-121719

Title: Surround suppression in area MT reduces the dependency of interneuronal correlation on tuning similarity

Authors: *L. D. LIU, C. C. PACK
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Abstract: Correlated noise in the responses of single neurons can strongly influence the signalling capacity of the neuronal population (Zohary et al. 94). Specifically, correlated noise

can either worsen or improve population decoding performance depending on how correlated noise varies with similarity of tuning between neurons ("signal correlation") (Averbeck et al. 06). In this study, we show that local surround suppression can reduce the dependency of correlated noise on tuning similarity, with important consequences for behavioral performance. We recorded from populations of MT neurons in monkeys performing a two-alternative, forced choice motion direction discrimination task. Stimuli were Gabor patches of various sizes. In agreement with human psychophysical results (Tadin et al. 03), we observed an apparent perceptual correlate of surround suppression: performance decreased with increasing stimulus size. In contrast, many MT neurons showed little or no surround suppression, and these neurons consistently outperformed the monkey on motion discrimination for larger stimuli. This suggests that perceptual performance is not due simply to a strategy of decoding from the neurons that are individually the most sensitive to the task parameters. A better account of the behavioral data was obtained by examining the responses of multiple, simultaneously-recorded MT neurons. We found that the dependency of correlated noise on tuning similarity between pairs of surround suppressed neurons (Pearson's correlation coefficient, $r=0.07$, $p=0.66$) was much smaller than that between pairs of neurons that lacked surround suppression ($r=0.77$, $p<0.001$). As a result, an ideal observer analysis (using a Support Vector Machine decoder) was able to extract more information from the population of surround-suppressed MT neurons, with the side effect of reduced performance for larger stimuli. Overall, these results suggest that surround suppression can effectively reduce the dependency of correlated noise on tuning similarity in sensory coding, and this reduction can account for the counter-intuitive aspects of behavioral performance.

Disclosures: L.D. Liu: None. **C.C. Pack:** None.

Poster

435. Decision Making and the Cortex

Location: Halls A-C

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Topic: D.04. Vision

Support: NIH EUREKA Award

Title: Correlated responses of v4, but not v1 neurons predict behavioral outcomes

Authors: *M. HU, A. PARAJULI, A. R. ANDREI, V. DRAGOI
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Abstract: The capacity of individual neurons to predict behavioral responses, or choice probability, has been extensively examined in sensory cortex. However, in addition to predicting behavioral outcomes, individual neuron responses encode incoming stimuli. The accuracy with which neuronal responses encode sensory information can possibly influence behavioral decisions. One major factor that influences the ability of neurons to accurately encode information is correlated activity. Despite this evidence, the relationship between choice probability and correlated activity is poorly understood. We performed simultaneous multi-electrode recordings in macaque areas V1 and V4 to investigate how trial-by-trial correlated variability (noise correlations) influences choice probability during a delayed match to sample task using natural stimuli. Animals were trained to detect a small difference in image orientation (3-5deg) between two consecutive stimuli that were presented 500-1200 ms apart. Over the course of 27 sessions, we recorded multiple single units from V1 (n=328) and V4 (n=72) in two monkeys (Macaca mulatta). Since the increase or decrease in neuronal responses to images were equally informative, we rectified choice probability to quantify the capacity of individual neuron to predict behavioral outcomes by defining the detection probability (DP). Despite the fact that most V1 and V4 neurons responded to the change in orientation between target and test stimuli, only 6.4% of V1 neurons and 13.8% of V4 neurons demonstrated statistically significant detection probabilities during the task. At the population level, we found a significant negative correlation between detection probability and noise correlations in V4 but not in V1 neurons. To further investigate the relationship between detection probability and noise correlations, we classified neurons into high DP and low DP groups based on the median of DP values within each session. During the test period, the high DP group of neurons in V4 had significantly smaller noise correlation values compared to the neurons in the low DP group. Surprisingly, we found that the high DP neurons had near zero noise correlations in incorrect trials. Finally, we found that the correlated activity of V4 neurons with the higher detection probabilities can be used to successfully predict behavioral decisions. Altogether, whereas correlated activity does not influence behavioral responses in V1, they play a significant role in area V4. Thus, correlations could act like an active switch to selectively control behavioral decisions in early and mid-level visual cortex.

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Poster

435. Decision Making and the Cortex

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Support: NSF-CRCNS IIS-0904430

CNS-103331

CIHR MOP-115178

CGSD-121719

Title: The relationship between choice-related variability of MT neuron responses and ongoing network activity

Authors: *Y. CUI[1], L. D. LIU[2], J. M. MCFARLAND[1], C. C. PACK[2], D. A. BUTTS[1]
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Abstract: Cortical neuron responses can be significantly different across repeated presentations of the same stimulus. The origin of such variability is unknown, but one common hypothesis is that it is due in part to differences in network states. In alert animals these differences might relate to the task and/or to the allocation of attention, independent of the stimulus. For example, during tasks involving perceptual decisions, the responses of cortical neurons are correlated with the subject's choice, even when there is no stimulus actually present. This is commonly referred to as "choice probability", which measures how well an ideal observer can predict the subject's choice using the neuron response. Here, we show that the correlation between neural responses and decisions can be predicted in part by network activity, as inferred from the local field potentials (LFPs). Specifically, we used a maximum-likelihood-based modeling framework that predicts the spiking responses using both the stimulus and LFPs. Spikes and LFPs were simultaneously recorded with a multi-electrode array from the middle temporal area (MT) of the monkey visual cortex during performance of a motion discrimination task or during passive fixation conditions. The results show that MT neuron responses are strongly linked to gamma oscillations (maximum at 35 Hz) as well as to lower-frequency delta oscillations (maximum at 2 Hz). Models that incorporated the LFP signal predicted a significant amount of non-stimulus driven response variability in passive conditions, explaining more about MT neuron responses than the stimulus itself. Moreover, in the context of the task, the model predicted a significant fraction of the choice-related variability. Further analysis revealed that the choice probability of a neuron is related to how well that neuron's phase preference in the delta-band aligned with the phases present during the decision period. These results therefore suggest that LFPs can be used to infer network activity and its influence on single-neuron firing and on perceptual decisions.

Disclosures: Y. Cui: None. **L.D. Liu:** None. **C.C. Pack:** None. **J.M. McFarland:** None. **D.A. Butts:** None.

Poster

435. Decision Making and the Cortex

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Topic: D.04. Vision

Support: Swartz Fellowship

Title: On the relationship between stimulus-evoked and choice-related responses and correlations during perceptual decision-making in a probabilistic inference framework

Authors: *R. M. HAEFNER[1,2]
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Abstract: In the traditional framework of perceptual decision-making, information on the retina is processed in a sequence of steps to give rise to our perception and inform decisions. As a result, the dominant framework for interpreting empirical observations like choice probabilities, response variability, and signal and noise correlations, is signal detection theory (Gold & Shadlen 2007). In this framework, empirically observed top-down influences like attention are interpreted as tuning this process by increasing stimulus information in sensory responses. We have previously shown how an alternative framework, probabilistic inference, can account for several findings that are hard to explain in the signal detection framework (Haefner et al. SfN 2013). Here, we i) generalize these results to make them independent of the details of how probabilities are represented by sensory neurons, ii) present a direct empirical test for whether signal detection or probabilistic inference provides a better model for perceptual decision-making, and iii) demonstrate how to use measured noise correlations to infer the internal model/strategy that the brain has learnt for a given task. Our findings rest on the fact that if sensory neurons correctly represent beliefs (or posterior probability distributions) then these beliefs will depend on both external information (observed variables) and relevant internal beliefs represented elsewhere in the brain. In the context of a decision-making task, a relevant internal belief about the correct choice. Bayes' rule relates p(choice | sensory variables) to p(sensory variables | choice), and hence, the bottom-up influence of the sensory neurons onto the decision neurons to the top-down influence of the decision neurons onto the sensory neurons. This leads to predictions for the relationships between stimulus-related quantity (e.g. tuning curves, signal correlations) and choice-related quantities (e.g. choice probabilities, noise correlations) that a) can be directly tested empirically, and b) are different from the predictions of the signal-detection framework. Finally, we show how to exploit this predicted symmetry

between bottom-up and top-down influences in order to decompose the noise correlations measured between sensory neurons during zero-signal trials into the 'principal components' of the internal model employed by the brain for the task, i.e. the way in which the decision-making areas interpret sensory responses. This provides a principled framework for tracking and understanding changes due to task learning, perceptual learning and attention.

Disclosures: R.M. Haefner: None.

Poster

435. Decision Making and the Cortex

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Support: ERC starting grant NEUROOPTOGEN

Bernstein Center Tuebingen funded by the German Ministry of Education and Research (BMBF; FKZ: 01GQ1002)

Title: Using sequential dependencies in neural activity and behavior to dissect choice related activity in V2

Authors: *H. NIENBORG[1], J. H. MACKE[2]
[1]Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Max Planck Inst. for Biol. Cybernetics; Bernstein Ctr. for Computat. Neurosci., Tuebingen, Germany

Abstract: During perceptual decisions the activity of sensory neurons co-varies with choice. Previous findings suggest that this partially reflects “bottom-up” and “top-down” effects. However, the quantitative contributions of these effects are unclear. To address this question, we take advantage of the observation that past choices influence current behavior (sequential dependencies). Here, we use data from two macaque monkeys performing a disparity discrimination task during simultaneous extracellular recordings of disparity selective V2 neurons. We quantify the sequential dependencies using generalized linear models to predict choices or spiking activity of the V2 neurons. We find that past choices predict current choices substantially better than the spike counts on the current trial, i.e. have a higher “choice probability”. In addition, we observe that past choices have a significant predictive effect on the activity of sensory neurons on the current trial. This effect results from sequential dependencies

of choices and neural activity alone, but also reflects a direct influence of past choices on the spike count on the current trial. We then use these sequential dependencies to dissect the neuronal co-variation with choice: We decomposed the choice co-variation of neural spike counts into components, which can be explained by behavior or neural activity on previous trials. We find that about 30% of the observed co-variation is already explained by the animals' previous choice, suggesting a "top-down" contribution of at least 30%. Additionally, our results exemplify how variability frequently regarded as noise reflects the systematic effect of ignored neural and behavioral co-variates, and that interpretation of co-variations between neural activity and observed behavior should take the temporal context within the experiment into account.

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Poster

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Spanish Ministry of Economy & Competitiveness,BFU2012-34838

Title: Evoked and mnemonic representation of spatial information in prefrontal cortex during memory-guided location comparisons

Authors: *P. REN[1,2], M. RAMON[3], P. M. SPINELLI[2], A. COMPTE[3], T. PASTERNAK[2]
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Abstract: We previously reported that neurons in dorsolateral prefrontal cortex (dlPFC) represent behaviorally relevant speed and direction of visual motion during all stages of memory-guided motion comparison tasks (Hussar & Pasternak, JN 2012, 2013). Here, we examined how spatial location is represented in dlPFC during an analogous memory-guided comparison task involving locations of motion stimuli. We analyzed dlPFC spiking activity and power spectra of local field potentials (LFP) during a task in which monkeys compared locations of two moving random-dot stimuli, S1 and S2, separated by a memory delay. Analysis of spiking activity revealed location selective responses during both S1 and S2 and these responses were

stronger during S2, most likely reflecting additional demands imposed by the comparison with the remembered location of S1. These observations were mirrored by LFP power in the β band of frequencies (12-30 Hz). β-band power showed significant location selective suppression during both S1 and S2, with stronger effects during S2. During the delay, many individual neurons showed gradual anticipatory activity modulation leading to the S2 onset, a pattern also observed in the β-band power of LFPs. In contrast to the close relationship between β-band power and spiking activity, θ-band LFP power showed task-dependent modulations dissociated from neuronal activity. Indeed, location selective delay activity in single neurons was transient and unrelated to the selectivity recorded during S1, appearing in different neurons at different times, a pattern analogous to that observed during the motion comparison tasks. However, LFP power in the θ-band (5-8 Hz) showed a strong enhancement during the memory, which was location selective and decayed by the end of the delay. These results reveal a striking parallelism between the way individual neurons represent sensory and spatial information during comparison tasks, suggesting that spatial location shares with the parameters of visual motion a common, or analogous neural substrate for representing sensory information in dlPFC during such tasks. This substrate might be different for evoked and mnemonic representations. Similar dynamics and location selectivity in β-band LFP and spiking activity suggest that evoked representations engage populations with clustered representations of sensory attributes, which oscillate at β frequencies. Mnemonic representations during the delay period, instead, may be supported by more distributed circuit-level representations linked to θ-band dynamics.

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Poster

435. Decision Making and the Cortex

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Support: HHMI

Berry Fellowship

FA9550-07-1-0537

Title: Changes-of-mind during decision-making: Neural correlates on single trials

Authors: *R. KIANI[1], C. CUEVA[2], J. REPPAS[3], W. T. NEWSOME[3,4]
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Abstract: Decision-making is a complex process in which different sources of information are combined into a decision variable (DV) that guides action. Neurophysiological studies have typically sought insight into the dynamics of the decision-making process and its neural mechanisms through statistical analysis of large numbers of trials from sequentially recorded single neurons or small groups of neurons. However, detecting and analyzing the DV on individual trials has been challenging. Here we show that by recording simultaneously from hundreds of units in pre-arcuate gyrus of macaque monkeys performing a direction discrimination task, we can predict the monkey's choices with high accuracy and decode DV dynamically as the decision unfolds on individual trials. This advance enabled us to study changes-of-mind (CoM) that occasionally happen before the final commitment to a decision. On individual trials, the decoded DV varied significantly over time and occasionally changed its sign, identifying a potential CoM. Interrogating the system by random stopping of the decision-making process at earlier times confirmed the validity of identified CoM's. Importantly, the properties of the candidate CoM's also conformed to expectations based on prior theoretical and behavioral studies (Resulaj et al., 2009): they were more likely to go from an incorrect to a correct choice; they were more likely for weak and intermediate stimuli than for strong stimuli; and they were more likely earlier in the trial. We suggest that simultaneous recording of large neural populations provides a good estimate of DV and explains idiosyncratic aspects of the decision-making process that were inaccessible before. References: Resulaj A, Kiani R, Wolpert DM, Shadlen MN (2009) Changes of mind in decision-making. Nature 461:263-266.

Disclosures: R. Kiani: None. **C. Cueva:** None. **J. Reppas:** None. **W.T. Newsome:** None.

Poster

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Title: Neural responses in parietal area MIP support a link between decision confidence and movement variability

Authors: *L. WOLOSZYN[1], K. K. ANANDALINGAM[2], R. VAN DEN BERG[2], D. M. WOLPERT[2], M. N. SHADLEN[1,3,4]
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Abstract: A decision is a categorical commitment to a proposition or plan of action. The all-or-none nature of commitment notwithstanding, we feel more confident about some decisions than others. Analogously, when reporting a decision with a reach, nominally identical movements to a choice target exhibit variability. Here, we explore the hypothesis that a component of this variability is related to confidence in the decision. We trained a rhesus monkey on a choice-reaction time (RT) task. The monkey reached towards one of two targets to indicate its decision about motion direction of a dynamic random dot stimulus. Difficulty was controlled by varying the percentage of coherently moving dots. As in previous studies, choices and RTs were well explained by the bounded accumulation of noisy evidence bearing on the direction alternatives. Although we did not interrogate the monkey about its confidence, recent studies have shown that confidence increases with motion strength and decreases with RT (e.g., slower decisions tend to be informed by less reliable evidence, leading to lower confidence). We exploited these regularities to seek out kinematic parameters that might reflect confidence. Like confidence, movement vigor (e.g., peak velocity) increased with motion strength and decreased with RT. Even after accounting for decision difficulty, movement vigor exerted significant leverage on the probability that the monkey would respond correctly, suggesting that kinematic variation reflects confidence. This inference is supported by data from human subjects who were instructed to report both their decision about motion direction and level of confidence. We discovered a putative neural correlate of the link between confidence and kinematics in the medial intraparietal area (MIP). Neurons in MIP have been shown to reflect the accumulation of evidence toward a decision in the motion task. We found that MIP firing rates accompanying decision formation predicted reach kinematics in a manner consistent with confidence. It thus appears that neural mechanisms underlying decision making lead not only to discrete choices but to graded manifestations of confidence as expressed through action. More generally, the results add to a growing body of evidence showing that a portion of variability in reaching movements can be attributed to a central, plan-related origin as opposed to a peripheral, execution-related one.

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Poster

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Burroughs Welcome Fund Career Award

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Fundacao para a Ciencia e Tecnologia

NIH-T-R01 1145850-2-PARJP

Title: Temporal certainty about stimulus presentation differentially affects response dynamics in dorsal premotor and primary motor cortex

Authors: *D. PEIXOTO[1,2], R. KIANI[3], C. CHANDRASEKARAN[4], K. V. SHENOY[4,5,6,7], W. T. NEWSOME[5,8]
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Abstract: In a dynamic environment subjects often integrate multiple samples of a signal and combine them before reaching a categorical decision. The combined information can be interpreted as a time-varying decision variable reflecting the current decision-state of a subject. We have previously reported that during a visual motion discrimination task dorsal premotor cortex (PMd) activity reliably predicts choice on single trials within 200ms of stimulus presentation (Peixoto, et al., SfN 2013). Moreover, prediction accuracy varies parametrically

with stimulus difficulty as expected for decision-related signals. Here we ask: 1) how choice predictive activity in primary motor cortex (M1) compares to PMd, and 2) how the dynamics of choice-related activity in each area are affected by temporal uncertainty about stimulus duration. We addressed the first question by using 'Utah' arrays to record simultaneously from several dozen neurons in PMd and M1 in two monkeys performing the discrimination task. We addressed the second question by comparing neural dynamics in a fixed duration task (1 s stimulus viewing period) vs. a variable duration task in which the stimulus duration was randomly varied between 0.1 and 1 s. The fixed duration task included a delay period after stimulus offset, which was eliminated in the variable duration task, thus placing a premium on quick, accurate decisions as soon as the stimulus was turned off. We assessed neural choice prediction by applying a logistic classifier to a 150 ms sliding window containing the activity of simultaneously recorded neurons in each area in both tasks. In the fixed duration task, choice prediction accuracy surpassed chance levels ~200 ms after stimulus onset for both PMd and M1. However, choice predictive activity in PMd soon exceeded that in M1 and remained greater for the rest of the stimulus presentation interval. At the time of the Go cue choice prediction accuracy was well above 90% and did not differ significantly between the two areas. In contrast, choice-predictive activity was faster and stronger when the duration of the stimulus was unpredictable, reaching 90% correct less than 500 ms after stimulus onset for both areas. This acceleration of choice-predictive dynamics was more striking for M1 than for PMd, raising the possibility that action signals are mixed with decision-related signals under conditions of temporal uncertainty. Future analysis of psychophysical kernels combined with stimulus coherence effects in PMd and M1 may enable us to pull these signals apart.

Disclosures: **D. Peixoto:** None. **R. Kiani:** None. **C. Chandrasekaran:** None. **K.V. Shenoy:** None. **W.T. Newsome:** None.

Poster

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Burroughs Wellcome Fund Career Award

Howard Hughes Medical Institute

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NIH-T-R01 1145850-2-PARJP

Title: Trial-by-trial covariation between PMd responses and action choice during a reaction time discrimination task

Authors: *C. CHANDRASEKARAN[1], D. PEIXOTO[2,3,6], W. T. NEWSOME[2,7], K. V. SHENOY[1,2,4,5]
[1]Electrical Engin., [2]Neurobio., [3]Sch. Of Med., [4]Program In Neurosci., [5]Bioengineering, Stanford Univ., Stanford, CA; [6]Champalimaud Neurosci. Inst., Lisbon, Portugal; [7]Stanford Univ. / HHMI, Stanford, CA

Abstract: Lesion studies propose that dorsal premotor cortex (PMd) is involved in the selection of actions on the basis of sensory cues. One framework suggests that in uncertain conditions, populations of PMd neurons would simultaneously represent potential actions. As disambiguating cues appear, the populations would compete until one population reaches a commitment state and results in an action (Cisek and Kalaska, 2005). This proposal makes a few predictions: 1) PMd responses should covary with the evolving choice; 2) the rate at which PMd responses diverge for action choices should correlate with cue ambiguity; 3) it should be possible to decode eventual choice of the animal from PMd responses on single trials; 4) the decode time course should correlate with reaction time (RT). We tested these predictions in a monkey performing a visual choice RT task and simultaneous recordings using U-probes. A monkey (T) used his right arm to report the dominant color in a central static checkerboard composed of isoluminant red and green squares. The percentage of red and green in the stimulus varied from trial to trial. While the monkey performed this task, we recorded small populations of single and multi-units from left PMd using 16 channel U-probes (27 sessions). Behaviorally, increases in stimulus difficulty led to more errors and slower RTs. We previously reported data consistent with the first two predictions (Chandrasekaran et al, SFN 13). A subset of the heterogeneous PMd neural population covaried with the evolving choice. Furthermore, the rate at which PMd responses diverged for eventual choices covaried with checkerboard difficulty. The new U-probe recordings confirmed these results. Here we leveraged U-probe recordings to test other predictions. We observed better than chance (> 50 %) decodes (linear discriminant, 200 folds, 70% training set) of eventual choice on single trials starting ~200 ms after stimulus onset. In addition, the time courses matched RT--- the rate at which the decoding performance increased was faster for shorter RTs and slower for longer RTs. In addition, consistent with a candidate decision variable, choice decode and its covariation with RT were observed within a stimulus difficulty. Preliminary recordings in putative M1 (n=11) suggested a different pattern than PMd. The rate at which M1 choice prediction accuracy increased was slower than PMd and in a few sites was only significant ~100-200 ms before movement onset. Our results demonstrate that in

ambiguous circumstances, responses in PMd covary on a trial-by-trial basis with the evolving action choice. Competition in PMd neurons may mediate the selection of the action to perform and when to start it.

Disclosures: C. Chandrasekaran: None. **D. Peixoto:** None. **W.T. Newsome:** None. **K.V. Shenoy:** None.

Poster

435. Decision Making and the Cortex

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Support: Sloan Research Fellowship

Title: Functional segregation of evidence accumulation and decision commitment in lateral intraparietal cortex

Authors: *R. L. GORIS, R. KIANI
Ctr. for neural science, New York Univ., New York, NY

Abstract: Perceptual decision making requires transforming sensory inputs into motor responses. This transformation is represented in the neural activity of sensory-motor areas in association cortex. For example, responses of lateral intraparietal (LIP) neurons reflect accumulation of evidence when the choice is communicated with an eye movement. Although accumulation of evidence is well established in LIP responses, the full range of computations for the transformation of evidence to choice is less clear. Here we shed light on the diversity of those computations by developing a novel doubly-stochastic model for spiking neurons. In our model, spikes are generated by a point process whose rate at each moment in time has a truncated Gaussian distribution. We studied the responses of 60 LIP neurons recorded from two monkeys performing a reaction-time direction-discrimination task. During decision formation, nearly half of LIP neurons are best explained as accumulators of noisy evidence over time: the variance of the underlying rate grows linearly with time and its autocorrelation drops with the square root of time. Response variability of the other half of neurons is best explained by trial-to-trial modulations of a canonical but stimulus-dependent response buildup. This group of neurons might represent a transformation of the incoming sensory evidence. Alternatively, they may represent the outcome of evidence accumulation across the population. Compatible with the

latter hypothesis, these neurons have a delayed representation of evidence accumulation and a weaker dependence on stimulus strength compared to the accumulator neurons. Moreover, they also exhibit an earlier pre-saccadic convergence of responses, indicating the decision criterion and the upcoming choice before the accumulating neurons. Finally, they have a faster return to baseline after saccade initiation, signaling disengagement from the decision-making process and the plan for a return saccade. Our results suggest that evidence accumulation and its readout for a final commitment to choice are represented by distinct neurons within the LIP network. Further, they support models in which a continuous readout of the accumulated evidence underlies the evolution of motor plans.

Disclosures: **R.L. Goris:** None. **R. Kiani:** None.

Poster

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Title: Prior probability of stimuli influences the computation of choice certainty

Authors: ***C. E. HATCH**, B. PURCELL, R. KIANI
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Abstract: Decisions are often accompanied by a sense of certainty--a graded and probabilistic assessment of an expected outcome. Although the behavioral and neural mechanisms of decision-making have been studied extensively, less is known about the mechanisms underlying choice certainty. Here, we investigate how manipulations of prior probability of stimuli in an environment influence certainty. Subjects viewed a patch of random dots and, when ready, reported the net direction with a saccadic eye movement to one of two direction targets. Task difficulty was manipulated by varying the percentage of coherently moving dots. After the first saccade, subjects wagered on the direction choice by making a second saccade to either a high-risk (HR) or low-risk target (LR). Choosing LR yielded a small monetary reward, regardless of the reported direction. Choosing HR yielded a larger reward, but only when the reported

direction was correct. Subjects performed the task in three blocks. In equal-prior blocks, the two motion directions were equally likely. In unequal prior blocks (right- or left-prior), one motion direction was three times as likely as the other. Consistent with previous reports, unequal priors increased the frequency of the more likely direction choice, shifting subjects' psychometric functions (PMFs; Hanks et al, 2011). Additionally, we found subjects shifted their certainty function (CF) by choosing HR more frequently after high-prior direction choices, and less frequently after low-prior choices, reflecting the integration of priors in the computation of certainty. However, changes in the CF deviated from a simple priors effect in two important ways: (i) CF shifts were smaller than PMF shifts, and (ii) the overall probability of choosing HR significantly increased compared to the neutral condition. The first deviation suggests that subjects suboptimally incorporated priors in the computation of certainty. The second deviation suggests that subjects' wagering behavior was influenced by a long-term expectation of increased accuracy with prior knowledge. Because both of these effects were robust to changes in the ratio of LR to HR value, they suggest changes in certainty rather than expected gain. Overall, subjects differentially incorporated prior probability information in their decision and the associated certainty. We suggest that the computation of certainty is influenced by both the sensory information within the trial as well as complex, long-term expectations based on prior knowledge about the environment.

Disclosures: C.E. Hatch: None. **B. Purcell:** None. **R. Kiani:** None.

Poster

435. Decision Making and the Cortex

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 435.16/GG13

Topic: D.04. Vision

Support: Howard Hughes Medical Institute

National Eye Institute grant EY11378

Title: The influence of the strength and variability of sensory evidence on confidence in a perceptual decision

Authors: A. ZYLBERBERG[1], *C. R. FETSCH[1], M. SIGMAN[2], M. N. SHADLEN[1,3,4]
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Abstract: The speed and accuracy of many simple decisions can be explained by bounded accumulation models in which noisy sensory information is integrated to a threshold, signaling the commitment to a choice. Such models imply a systematic relationship between the state of accumulated evidence (termed a decision variable, DV) and the probability - contingent on elapsed decision time - that a choice based on that evidence will be correct. This relationship could allow the brain to establish a level of confidence in the decision, thereby uniting the three pillars of choice behavior (accuracy, reaction time, and confidence) under a common framework. A counterintuitive prediction of bounded accumulation models (and others based on signal-detection theory) is that increasing the variability of momentary evidence ('noise') leads to higher confidence despite lower accuracy. The basic insight is that with greater noise, the DV tends to drift further away from the neutral level, which ordinarily is associated with stronger evidence and thus greater confidence. Support for this prediction comes from causal manipulations of visual cortex in both humans and monkeys; however, the mechanism by which these manipulations increased noise is unclear, and the small amount of added noise was not well controlled. Here we addressed these issues by manipulating both the average strength of evidence and its variability. Monkeys reported the perceived direction of motion of a dynamic random-dot display. We controlled the difficulty of the decision by varying the motion strength (percent coherence) and viewing duration. On half of the trials, the monkey was offered the option to withhold its report of the motion decision and obtain a small but certain reward. This type of 'post-decision wager' has been shown to reveal the animal's confidence in the binary direction choice. In addition to varying the motion strength across trials, we mimicked the addition of noise by resampling the coherence from frame to frame within a trial. This novel visual manipulation confirmed the paradoxical increase in confidence for stimuli of lower reliability, despite a decrease in accuracy. In separate experiments, we found that the same manipulation led to faster reaction times, implying that the additional noise pushed the DV to a bound more quickly. Analyses of motion energy and identical stimulus repetitions yielded insights into the relative contribution of internal vs. external (stimulus-driven) noise. Together, the results suggest that the mechanism underlying confidence judgments does not have direct access to the reliability of sensory evidence, but instead relies on the state of an evolving decision variable.

Disclosures: A. Zylberberg: None. **C.R. Fetsch:** None. **M. Sigman:** None. **M.N. Shadlen:** None.

Poster

435. Decision Making and the Cortex

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Topic: D.04. Vision

Support: NIH Grant T32EY007136

Alfred P Sloan fellowship

Title: Adaptive decision-making: a role for choice certainty in updating environmental belief

Authors: *B. PURCELL, R. KIANI
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Abstract: Our decisions are guided, in part, by a belief about the current environment - the context that defines a set of associations between stimuli, actions, and outcomes. To accommodate dynamic changes in the environment, we must flexibly adapt our belief about the current context based on feedback and prior expectations. For example, a confident choice that does not produce the desired outcome signals a change in the environment. We developed a novel task to study how choice certainty - the expectation that our choices will produce a desired outcome - is utilized to adapt future behavior. Human subjects viewed a patch of stochastic moving dots and reported the net direction (right or left) by choosing one of four targets with a saccadic eye movement. The targets were arranged in two pairs that represented two distinct environments - one pair above fixation and another below. The active environment stayed fixed for several trials and then switched without any explicit cue to the subject. The subject received positive feedback only if the chosen target represented both the correct motion direction (left or right) and the active environment (above or below fixation). Negative feedback, however, could arise from choosing the wrong environment or motion choice. To update their environment choices following negative feedback, subjects resolved this ambiguity by utilizing motion-choice certainty. They were more likely to switch environments after they received negative feedback on trials in which motion strength was higher and the stimulus duration was longer. The influence of choice certainty on future environment choices persisted across multiple trials, indicating that subjects integrated certainty across trials to update their belief about the active environment. Our results suggest that the behavior was guided by two principles: computation of certainty about motion direction within trials and integration of certainty and feedback across trials. A model that (i) used choice certainty and feedback to compute evidence for an environmental change, and (ii) integrated this evidence to a criterion to switch the behavior, successfully explained subjects' choices in our task. Our results provide a framework for understanding long-term integration of past expectations and feedback to adapt to dynamic changes in the environment.

Disclosures: **B. Purcell:** None. **R. Kiani:** None.

Poster

435. Decision Making and the Cortex

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Topic: D.04. Vision

Support: NIH Grant R01H047520

Title: Dorsal-ventral visual stream structural integrity and functional connectivity predict 6-year longitudinal growth in math skills

Authors: ***T. M. EVANS**, J. KOCHALKA, T. J. NGOON, C. J. BATTISTA, V. MENON
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Abstract: Early math proficiency lays the foundation for future academic success and for numerical skills essential in today's technological society; identification of cognitive and neural markers associated with weak long-term outcome has therefore taken great urgency. Previous research has shown a relationship between individual differences in math skills and the structural integrity of brain regions known to support numerical processing. It is currently not known whether structural brain measures can predict individual children's gain in mathematical abilities over time. Here we use a longitudinal design to investigate whether changes in mathematical abilities in children between the ages of 8 to age 14 are related to brain structure or cognitive abilities at age 8. Interestingly, we found that gray matter volume in the left intraparietal sulcus and fusiform gyrus at age 8 predicted subsequent gains in math skills. Furthermore, intrinsic functional connectivity between these two regions was also predictive of individual behavioral gains. Crucially, cognitive measures including IQ, working memory, and reading at age 8 did not predict longitudinal changes in math ability. Our study provides the first evidence that the structural integrity of both dorsal and ventral stream brain regions as well as their functional coupling influence long-term acquisition of basic math skills in children, and highlights potential biomarkers for identifying children who might benefit most from educational intervention.

Disclosures: **T.M. Evans:** None. **J. Kochalka:** None. **T.J. Ngoon:** None. **C.J. Battista:** None. **V. Menon:** None.

Poster

436. Sensorimotor Transformation: Behavior

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Program#/Poster#: 436.01/GG16

Topic: D.05. Visual Sensory-motor Processing

Support: Jeffress Foundation

Title: Determinants of the escape response of crickets to looming stimuli

Authors: *A. M. CHILDS, K. L. REIMAN, C. R. EBEL, C. L. CLELAND
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Animals respond to aversive stimuli with escape or withdrawal responses. In crickets, wind, which might normally be produced by an approaching predator, has been shown to evoke an escape response in which the cricket turns and then runs or jumps away. Looming stimuli, however, better approximate the combined stimulus modalities (wind, vision and sometimes touch) associated with attack by a predator. Nevertheless, there are a limited number of studies on the response of crickets to looming objects. The goal of this study was to describe the escape response of the cricket (Acheta domesticus) to looming stimuli delivered from each of 8 angles around the cricket. Looming stimuli were created by attaching a 3" black polyurethane ball to the end of a 12" air cylinder (45 degrees to vertical) driven by compressed air at a speed of about 90 mm/sec. The direction of "attack" was varied in 45 degree increments around the cricket. The cricket's response was recorded by a high-speed video camera (Redlake/IDT, 650 fps) placed overhead. The top of the head, thoracic-abdominal junction and the tip of the abdomen were tracked over time (Proanalyst, Xcitex) to provide the two dimensional locations and orientation of the abdomen and the head/thorax. Further, the initial locations of the tip of tarsi just prior to movement were recorded. In response to looming stimuli, crickets typically first pointed their proximal antenna toward the looming object and then initiated a turn away from the stimulus. At the completion of the turn, the crickets either walked (89%) or jumped (11%) away. The direction of the turn was almost always (98%) away from the stimulus. Further, the response direction varied with the laterality of stimulus (slope = -0.57; 1.0 would be directly away from the stimulus; $p<0.0005$). Interestingly, the head/thorax nearly always led the turn. These results demonstrate that the direction of the crickets' escape turn from looming stimuli depends strongly on both the side and the laterality from which the stimulus is delivered.

Disclosures: **A.M. Childs:** None. **K.L. Reiman:** None. **C.R. Ebel:** None. **C.L. Cleland:** None.

Poster

436. Sensorimotor Transformation: Behavior

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 436.02/GG17

Topic: D.05. Visual Sensory-motor Processing

Support: Jeffress Foundation

Title: Determinants of the escape response of crickets to localized heat stimuli

Authors: *B. MITCHELL, S. C. HEITSCH, G. W. REBHUN, E. G. THOMSON, C. L. CLELAND
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Animals respond to aversive stimuli with escape or withdrawal responses. In crickets, wind or looming stimuli, which might normally be produced by an approaching predator, are commonly used to evoke an escape response in which the cricket turns and then runs or jumps away. Although in mammals aversive heat stimuli have been used routinely to evoke nociceptive withdrawal responses, there have been no studies of the cricket's response to localized heat stimuli. The goal of this study was to describe the escape response of the cricket (Acheta domesticus) to heat stimuli delivered to each of its six tarsi and determine the factors that control direction and magnitude of the response. Heat was delivered to the tarsus of each leg in 25 crickets with an infrared laser diode (980nm) and the response was recorded by high-speed video (Redlake/IDT, 650 fps) placed overhead. The top of the head, thoracic-abdominal junction and the tip of the abdomen were tracked over time (Proanalyst, Xcitex) to provide the two dimensional locations and orientation of the abdomen and the head/thorax. Further, the initial locations of the tarsi just prior to movement were recorded. In response to heat stimuli, crickets first retracted the stimulated tarsal, then turned by pivoting about a point toward the rear of the animal, and finally either walked (86%), jumped (9%) or remained largely stationary (5%). As with wind or looming stimuli, the turn was always away from the location of stimulus. In contrast, however, jumping was less frequent than with the other types of stimuli. The rotation of the head/thorax matched the rotation of abdomen, unlike crickets' response to looming stimuli in which the head leads the abdomen. These results demonstrate that crickets escape from heat as well as from looming, touch and wind stimuli, and offer the opportunity to identify common movement strategies by comparing the escape responses to the four different stimuli.

Disclosures: B. Mitchell: None. **S.C. Heitsch:** None. **G.W. Rebhun:** None. **E.G. Thomson:** None. **C.L. Cleland:** None.

Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant DP1 NS082121-02

Title: Behavioral characterization of ultraviolet light avoidance in the larval zebrafish

Authors: *D. A. GUGGIANA-NILO, F. ENGERT
Harvard Univ., Cambridge, MA

Abstract: Color vision research has made immense advances in determining the pathways followed by color information from the retina into the thalamus and then into the visual cortex. The only lacking component in these findings is a comprehensive description of the system in terms of topography, since it is known that visual systems are often wired in a somewhat organized manner, usually following some degree of mapping to real space. The zebrafish larva (Danio rerio) offers a unique platform to topographically characterize the chromatic component of the retinofugal projection, since its transparency coupled with the ability to make transgenics expressing genetically encoded calcium indicators permits non-invasive 2-photon calcium imaging of many neurons simultaneously. Additionally the larval zebrafish retinofugal projections are all accessible and contain similar response properties and clustering as the mammalian visual system.In this study we chromatically characterize the avoidance of ultraviolet (UV) light observed in the larval zebrafish. This is an unusual behavioral response since zebrafish larvae are attracted to white visible light within a wide range of intensities (phototaxis). Since the zebrafish has a tetrachromatic retina with UV sensitivity in addition to red, green and blue, we measured its 4-color behavioral spectral sensitivity function based on a previously reported phototaxis paradigm. In brief, we used a closed-loop projection system to present different colors at each side of the fish and force a choice of turning direction during each frame. The system allowed us to change light intensity and hue dynamically over the course of the experiment. Our data supports previous observations of equivalent processing of all the visible light color channels available to the fish, with attraction at medium and low levels and avoidance at very high levels. Instead, UV light was avoided at all light levels. Additionally, the strength of the response depended directly on the UV light level used. This response was less intense when UV light was presented concomitantly with visible light, but it was still observed. As our next step the behavioral studies will then be complemented with functional imaging studies using multi-photon microscopy in combination with fluorescent protein-based calcium

indicators. This will allow us to characterize the retinofugal projection in a color-dependent manner while the same visual stimuli used in the behavioral experiments are presented.

Disclosures: D.A. Guggiana-Nilo: None. **F. Engert:** None.

Poster

436. Sensorimotor Transformation: Behavior

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Program#/Poster#: 436.04/GG19

Topic: D.05. Visual Sensory-motor Processing

Title: Biased visuomotor behavior suggests a simple computation underlying amphibian target tracking

Authors: *W. R. MOWREY, A. LEONARDO
Janelia Farm Res. Campus, Ashburn, VA

Abstract: Visual object tracking is critical to many behaviors, yet how downstream brain areas use retinal output to accomplish this remains unclear. While many studies have explored the possibility of the brain optimally reconstructing a visual stimulus [1,2], recent work suggests a prominent role for sub-optimal computations that are simple but effective [3]. Here we further explore this in the context of shape discrimination. A linear model of post-retinal processing, based on a population vector average (PVA) of Off-center ganglion cells, predicts a shape-dependent bias in target tracking. This bias arises because Off-cells track the leading edge of a moving object, shifting the position estimate forward for targets with a body axis elongated in the direction of motion, but not for targets elongated in the orthogonal direction. In contrast, an optimal model could account for the retinal circuit dynamics underlying this asymmetry and achieve shape-invariant tracking. To test these ideas, we presented toads (Anaxyrus terrestris) with artificial targets elongated parallel or perpendicular to the motion direction. In all cases tongue projections were consistent with the predictions of the PVA model. We suggest this circuit provides important context for understanding the classic worm/anti-worm observations of Ëwert et al. [4]. The PVA tracking model further predicts specific contrast-sensitivity in behavior. PVA tracking is robust to contrast magnitude, with performance only degrading abruptly at low contrasts. However, the Off-cell-based PVA is acutely sensitive to the sign of contrast: targets darker than the background drive leading edge tracking, while those lighter are tracked by the trailing edge. We tested these predictions by presenting toads with targets of varying luminance on a uniform gray background. Consistent with the PVA model, tongue

projection accuracy was insensitive to contrast magnitude, and tongue projections were absent in the low-contrast regime. However, toads also showed a strong preference for targets darker than background, and rare tongue projections toward targets lighter than background were still directed toward the leading edge. These observations are consistent with a PVA model where tracking is dominated by either Off- or On-cell input, depending on target contrast. Future work will investigate how Off and On pathways can flexibly contribute to tracking performance. [1]DOI:10.1126/science.2063199 [2]DOI:10.1523/JNEUROSCI.3305-05.2005 [3]DOI:10.1523/JNEUROSCI.2257-13.2013 [4]DOI:10.1016/j.bbr.2011.03.031.

Disclosures: W.R. Mowrey: None. **A. Leonardo:** None.

Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Support: Grant/Other **Support:** Marie Curie Incoming International Fellowship (WHISKERATTENTION project, grant number: PIIF-GA-2012-331122)

Title: Visual backward masking in rats: A behavioral task for studying the neural mechanisms of visual awareness

Authors: *M. WATANABE[1], N. TOTAH[1], K. KAISER[2], S. LÖWE[2], N. K. LOGOTHETIS[1]
[1]Logothetis, Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany; [2]Univ. of Tuebingen, Tuebingen, Germany

Abstract: The neural mechanism of visual awareness has been primarily studied by contrasting neural activity between visible and invisible stimuli, in attempt to unveil the necessary and sufficient condition for neural representations to enter conscious vision. Visual illusions that render stimuli invisible (e.g., binocular rivalry, backward masking) are prominent behavioral paradigms. So far, majority of studies on visual awareness have been conducted on human and non-human primates. Although these studies greatly contributed to establishing specific brain region-dependent modulation of neural activity by awareness, the field would benefit from being able to conduct experiments on rodents. This advance would provide access to modern techniques such as optogenetic manipulation and two-photon imaging, etc. Here, for the first time, we report backward masking in rats. Backward masking is a visual illusion in which a

target is rendered invisible by a visual mask that follows the target with a brief stimulus onset asynchrony (SOA). We first developed a head-fixed rat spherical treadmill system that is amicable to rats performing visual tasks with low contrast, short duration stimuli, which are required for testing backward masking. Rats were initially trained to discriminate a "go" target (vertical grating: 0.15cpd, 28deg visual angle) and a "no-go" target (horizontal grating: 0.075 cpd, 28deg visual angle) without the visual mask. They responded either by running or staying still on the treadmill during a brief time-window after stimulus presentation and were rewarded with drops of water for running in response to a "go" target and punished with time-out penalty for running in response to a "no-go" target. Duration and contrast of target stimuli were gradually reduced to experimental parameters for the backward masking experiment (duration:16ms, luminance contrast:15%). After achieving threshold performance (d' >1.5), backward masking experiments were conducted with SOAs at 16, 33, 49, 66, 83, 99, 116 ms. Plaids were used as visual mask (duration:33ms, luminance contrast:95%). In all 5 rats, smaller SOA led to statistically non-significant differences between hit and false-alarm ratio. In contrast, difference between hit and false alarm rate were significant for larger SOAs. Threshold SOAs at which masking occurred varied across rats (range: 33m -66ms). In conclusion, a visual stimulus can be rendered invisible with short SOAs, and hence, backward masking can be used to study the neural correlate of consciousness in rats.

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Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Support: IDeA CTR support – NIH/NIGMS Award Number U54GM104942

Title: Insights from modeling the process of learning a pair of similar visuomotor tasks in rats

Authors: *R. ELLISON[1,2], A. POLLARD[2], M. PRIESTAS[2], S. YAKOVENKO[2]
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Abstract: The energetic cost of maintaining neural substrate is an important parameter that optimizes the development of new and the adaptation of preexisting neural pathways. This

process participates in the advantageous expansion of behavioral repertoire. Georgopoulos & Grillner (1989) posed a hypothesis that control circuitry of the motor cortex and cerebellar areas is shared for similar visuomotor behaviors such as reaching and locomotion. Thus, for a pair of reaching and locomotor tasks the overlap in learning dynamics indicates the degree of overlap among shared control circuitry. To test this hypothesis we utilized 11 Sprague-Dawley rats trained to perform two visuomotor tasks. For the locomotor task, rats were trained to walk on an enclosed treadmill for a 10 min session. The training of consistent stepping was done by ramping the treadmill speed to the rat's preferred speed associated with minimum sideways deviations and stops that were also discouraged by mild electrical stimulation. The assessment of performance was based on parameters evaluating the consistency and type of gait, severity of counterproductive behaviors and the number of stimulation events per session. In the reaching task, the rats were trained to reach and grasp flavored food pellets through a slot in a plexiglass cage during a 25-30 min session following or preceding the locomotor session on the same day. The number of successful and unsuccessful retrieval attempts per straight, right and left reaches indicated the performance during this task. To analyze data further, the learning process was modeled with leaky integration dynamics similar to that of Smith et al. (2006). We found a significant relationship among successful pellet retrieval attempts between forelimbs indicating skill transfer. The simulations supported this finding showing strong relationships between learning gain and skill leak parameters of the model. However, when we applied a linear regression model and the skill acquisition model to test the skill transfer between the reaching and locomotor tasks, we found no relationship. These findings support the idea of separate neural circuitry responsible for the performance in the two tasks, suggesting non-overlapping learning processes in premotor networks. However, the execution pathways may still be shared for these tasks.

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Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Support: 1R01HD071978-01A1

Title: How does variability in tactile feedback affect adapatation of grip forces to surface friction?

Authors: *P. RAGHAVAN[1], S. BILALOGLU[1], D. GELLER[1], V. ALURU[1], J. RIZZO[1], Y. LU[2]
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Abstract: People grasp objects of various textures with ease by adapting their fingertip (grip) forces to the friction at the grip surface. Adaptation occurs by first sensing the grip force necessary to prevent slippage of the object, and then using this information to modify the rate of change of grip force (grip force rate) to the frictional surface, where smoother objects elicit higher grip force rates than rougher objects. However moisture at the fingertip can influence the grip force variably - it may increase in some individuals and decrease in others. This introduces noise into the system and may influence the rate at which adaptation occurs. We tested the conditions under which optimal adaptation of fingertip grip forces to friction at the object surface occurs in neurologically intact individuals. Subjects grasped and lifted objects with 16 different frictional surfaces in blocked trials under three conditions: using barehands, with a thin plastic film on the fingertips, tegaderm (to control for moisture at the grip surface interface), and with a layer of foam and tegaderm (to reduce tactile sensibility). The weight was constant across the trials. The order of presentation of the frictional surfaces for each condition and the order of the conditions were randomized across subjects to preclude an order effect. We found that the peak grip force rate, which reflects fingertip force adaptation, did not differ between barehands and tegaderm, but was significantly higher for foam across the practice trials. Interestingly, the static grip force and the safety margins (difference between the minimal force required and the actual grip force at lift) were higher for barehands than tegaderm and significantly higher for foam, suggesting that tegaderm does not reduce grip force efficiency. The correlation between the coefficient of friction and the peak grip force rate was highest for tegaderm followed by barehands, and lowest with foam. We also found that the variability in peak grip force rate during the last three trials was significantly lower for tegaderm compared with barehands. The results suggest that moisture in barehand trials may introduce variability in tactile feedback from the fingertips, which influences adaptation of fingertip forces to surface friction; this can be effectively controlled using tegaderm. A foam coating on the fingertips can reduce tactile sensation sufficiently to impede adaptation of fingertip forces to surface friction. Wearing a thin layer over the fingers, such as surgical gloves, may produce more efficient and consistent grip force performance over a wide range of textures.

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Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Title: Comparison of static and dynamic point-to-point tasks for visuomotor adaptation

Authors: *A. HANTJIS, S. PARKER, J. LAWRENCE-DEWAR
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Abstract: The ability to correct hand movements is essential for successfully completing simple day-to-day tasks. While our interactions with stationary targets have been well studied, visuomotor adaptation behaviour towards moving targets has received less attention. We hypothesized that a dynamic task that permits multi-directional movements would lead to better adaptation than a typical static task. The present study compares participants' behaviour during a computer-based point-to-point visuomotor adaptation task to a novel task in which the target is moving. Twenty-two young, healthy, right-handed adults (11 male) used a MRI-compatible trackball to control the movement of a cursor to a target during four conditions (normal, flip in x axis, flip in y axis, and flip in both axes). Five hundred trials were divided into two sets (static and dynamic target types). To account for order effects, participants were divided into two groups so that one group received the static target trials first while the other group completed the dynamic target trials first. Each set was divided into four "phases" to examine changes in behaviour: a pre distortion phase (first 50 trials with normal cursor control), an early distortion phase (first 75 trials with all cursor conditions pseudorandomized), late distortion phase (next 75 trials with all cursor conditions pseudorandomized), and a post distortion phase (last 50 trials with normal cursor control). A repeated measures ANOVA was performed using a 2 (target type) x 2 (group number) x 2 (phase) factorial design to examine significant differences in mean measures of path distance, movement time, and cursor velocity between the static and dynamic tasks. Improvements in adaptation were observed in both target types reflected by decreases in path distances between the early and late distortion phases. Additionally, decreased movement times and increased cursor velocities were observed between the pre and post phases during dynamic target trials. Since a plateau was seen in the second half of the task, it was suspected that data from the first half would better represent which target type lead to better adaptation. Participants showed better adaptation during the dynamic task reflected by faster cursor velocities between the pre and post distortion phases than during the static task. Likewise, shorter path distances and movement times were observed between the early and late distortion phases.

The results of this study provide a framework for future investigations of the neural correlates of visuomotor adaptation through functional MRI studies.

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Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

Title: Learning joint actions: Cooperation strategies

Authors: ***J. D. KLEIN**, S. HELMS-TILLERY, C. BUNEO
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Abstract: Joint actions involve two or more agents coordinating their behavior in space and time to perform a particular task. Despite the fact that these types of actions are very commonly performed in everyday life, little is known about how joint actions are learned and arise from interaction between subjects. Here we addressed how joint motor actions are learned in the absence of direct visual, auditory and haptic cues regarding another agent. Seven pairs of human subjects (dyads) learned a virtual reality based joint action task. The task involved moving a cursor from the center of a visual display outward to one of four peripheral targets, and returning to center within a two second time period. Motion of the cursor depended on the arm movements of both subjects, i.e. the mean of their simultaneous (but not necessarily synchronous) individual actions. Subjects were provided with cursor feedback, but visual cues of the moving arms and other body parts were withheld. Subjects were also instructed not to provide auditory cues to one another. However, auditory cues indicating trial success/failure were provided. In this task, the mapping between subject motion and cursor motion was not always veridical but involved a visuomotor rotation of 30 degrees that was applied to either one or both subjects in separate blocks. Subjects also performed an initial control block and three intervening de-adaptation blocks where no rotation was applied. Each block required the successful completion of 15 trials per target. Success rates during each block were calculated to quantify each dyad's performance. Performance across dyads was highly variable and included groups with relatively high success rates (>75%) and relatively low success rates (<60%) with no groups falling between these extremes. Additionally, two strategies were identified based on the relative magnitudes of the performed movements (i.e. path lengths). Some dyads performed the task using largely similar

path lengths while others appeared to engage in a motor domain equivalent of "social loafing", where one participant's paths were much shorter in length than their partner's. In some dyads this difference was detrimental to performance, but in one group it appeared to be a highly successful strategy. The results are largely consistent with previous studies of joint action, which have identified both cooperative and competitive strategies depending on the context in which a task is performed. Future work will seek to identify hallmarks of these strategies using additional variables in both the spatial and temporal domains.

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Poster

436. Sensorimotor Transformation: Behavior

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Topic: D.05. Visual Sensory-motor Processing

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Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Reciprocal coupling of visuo-motor linkages synchronize interpersonal postural coordination

Authors: *S. OKAZAKI[1], T. KOIKE[1], M. HIROTANI[2,1], J. BOSCH-BAYARD[3], H. K. TAKAHASHI[4], M. HASHIGUCHI[4], N. SADATO[1,4]
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Abstract: In social settings, interpersonal behavioral coordination is achieved accurately without verbal communication and often shows unintentional synchronization in both timing and form. This has been explained by the self-organization of two oscillating systems. However, it is still unclear how non-rhythmic motions, which are usually seen in social settings, are precisely synchronized without delay. Automatic mimicry, the spontaneous copying of the low level kinematic features of action, is ubiquitously observed in social setting. Automatic mimicry is based on the tight linkage between perception and motor representations and could be regarded

as causal influences from the observed to the observing persons, the latter of which inevitably follows the former. However, what happens if the causal influences are reciprocal? We hypothesized that reciprocal feedback of the motion signal is essential for lag-0 synchronization of non-rhythmic motions. We investigate the postural control of two persons standing upright and facing each other. The pair was regarded as a feedback system in which each of the postural wobbling is causal to the other represented as a tendency of automatic mimicry. A total of 44 participants were recruited and their postural wobbling was measured by the motion capture system. Pair of participants stood upright face to face with and without blindfold. This was to generate reciprocal (OO), unidirectional (OB/BO), and isolated (BB) conditions of visual interaction. First, correlation of the time series of the postural wobbling was calculated. Second, using bivariate autoregressive (AR) model, we calculated the noise contribution ratio (NCR) which is an index of the causal influences between the pair. Finally, we simulated the pair-wise non-rhythmic synchronization based on the AR model. We found that the postural wobbling during OO condition showed lag-0 synchronization between participants whereas during OB/BO conditions sighted person followed their blindfolded partner by 300 to 400 msec. During BB condition, there was no synchronization, indicating that the causal influence is mediated by vision. Correspondingly, the causal influence by means of NCR to the sighted participants significantly larger than that to the blindfolded. During OO condition, the pair specific time delay of the synchronization was correlated with the differences of the causal influences. Finally the simulation replicated the relationship between the time delay and the causal influences. These results suggest that the tendency of automatic mimicry of equivalent size is essential for lag-0 synchronization of unintentional non-rhythmic motions.

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Poster

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Program#/Poster#: 436.11/GG26

Topic: D.05. Visual Sensory-motor Processing

Title: Optical flow with roll oscillations affects postural control during human locomotion

Authors: ***J. PICKHINKE**, T. RAND, D.-J. EIKEMA, M. MUKHERJEE
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Abstract: Postural control relies on input from different sensory systems. In particular, the visual system is essential for optimal postural control during static standing. However, visual contributions to postural control while walking have yet to be determined conclusively. Moreover, the use of virtual reality environments has proven to be an effective tool for determining visual influence on human movement. It is known that both frequency and velocity of a tilting visual scene can induce a standing postural response, and the degree with which frequency and amplitude had an effect was dictated by the frequency range of the stimulus. Despite this, it is unknown whether frequency or amplitude of an oscillating optical flow affects locomotion. In this study, we investigated whether medial-lateral (roll) oscillations of different frequency and amplitude combinations embedded within a natural speed-matched anterior-posterior (AP) optical flow would affect walking postural control. The dependent variable was the amount of medial-lateral drift exhibited by a reflective marker positioned at the C7 vertebrae of participants (drift was calculated using the range between the maximum excursions in both directions along the x-axis). Healthy young adults walked under ten optic flow conditions. The control condition with no oscillation was compared to nine conditions (3 frequencies - 0.1Hz, 0.3Hz, 0.5Hz; 3 amplitudes - 1°, 5°, 10°). At each condition, participants walked at their preferred walking speed which was matched to their optic flow speed. Results showed a significant main effect of frequency ($F_{2,20} = 3.957$, $p = 0.036$). This effect resulted from a significant reduction in excursion from 0.1Hz to 0.3Hz. A significant effect of amplitude ($F_{2,20} = 20.319$, $p < 0.001$) was seen as well. The excursion increased significantly as the amplitude increased from 1° to 5° and 1° and 10°. There was no significant increase in excursion when progressing from 5° to 10°. We also found a significant interaction effect ($F_{4,40} = 2.686$, $p = 0.045$). This was caused by a much larger increase in excursion from an amplitude of 5° to 10° at a frequency of 0.3Hz in comparison to the other two frequencies. In conclusion, the amplitude of an oscillating optical flow affects medial-lateral excursion more strongly than frequency. However, at a particular frequency threshold this effect may be reversed. Therefore, visual control of ML motion during gait depends on both the amplitude and frequency of the visual feedback concurrently. Although, such an effect also depends on a frequency threshold.

Disclosures: **J. Pickhinke:** None. **T. Rand:** None. **D. Eikema:** None. **M. Mukherjee:** None.

Poster

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Program#/Poster#: 436.12/GG27

Topic: D.05. Visual Sensory-motor Processing

Title: Motor control deficiencies contribute to impaired reactive driving performance in older adults

Authors: *C. KIM, H. MOON, L. JECK, T. ONUSHKO, N. LODHA, E. CHRISTOU
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Abstract: There are currently 35 million drivers over the age of 65 in the US. Age-related decline in visual information processing and motor control significantly increases the risk for automobile accidents in older adults. The majority of automobile accidents occur during reactive driving situations where the drivers must respond to visual stimuli with quick and precise movements. The purpose of this study was to compare the reactive driving performance in young and older adults. We compared reactive driving performance in 12 young (22.8 ± 3.7 yrs; 5 females) and 20 older adults (71.3 ± 7.9 yrs; 10 females). Subjects controlled the gas pedal during a visuomotor task and responded to a sudden visual stimulus by moving their right foot from the gas pedal to the brake pedal. Their goal was to apply a 40 N force on the brake pedal as quickly and as accurately as possible. Brake pedal control was quantified with the brake force error and variability (CV in percentage). The response to the visual stimulus was quantified with the pre-motor response time (from stimulus onset to tibialis anterior activity onset) and motor response time (onset of tibialis anterior activity to onset of the brake pedal force) and total response time was quantified with summation of pre-motor and motor response time. Older adults exhibited longer total response time to the visual stimulus (866.1 vs. 771.2 ms, P=0.014). The pre-motor response time was not significantly different for young and older adults (493.8 vs. 448.8 ms, P=0.09), whereas the motor response time approached significant difference (372.3 vs. 322.4 ms, P=0.06). Compared with young adults, older adults exhibited greater brake force error (22.2 vs. 10.9 N, P=0.007) and greater brake force variability (23.3 vs. 14.3 %, $P < 0.001$). These findings suggest that older adults exhibit impaired reactive driving performance relative to young adults likely due to deficits in motor control than visual information processing.

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Poster

437. Reaching Action

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Topic: D.05. Visual Sensory-motor Processing

Support: Canadian Foundation for Innovation

Title: Binocular advantage in prehension movements performed in visually stimulating environments

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Abstract: Background: Vision provides important sensory input for the control of upper limb movements, especially during manipulation tasks that involve reaching and grasping objects. The purpose of this study is to examine the role of binocular vision during a prehension task in a visually stimulating environment where the target object was embedded among distractors. Methods: Thirteen adults reached and grasped for a cylindrical peg while eye movements and reach kinematics were recorded. The complexity of the visual environment was manipulated by varying the number of distractors and by varying the saliency of the target (i.e., salient target (pop-out), conjunction target, salient distractor). There were three viewing conditions: binocular, left and right monocular, which were randomized in blocks between participants. Results: There are 3 main findings: 1)Participants made significantly more errors and picked up the wrong peg during monocular in comparison to binocular viewing (5.5% vs. 2.8%, respectively). During monocular viewing the number of errors increased for trials with a larger set size and for invalid and conjunction trials. These manipulations had no significant effect on error rates during binocular viewing. 2)Binocular vision provides a greater advantage for movement planning. Higher peak acceleration (4.6 m/s2 vs. 4.1 m/s2, $p<0.05$) and peak velocity (binocular: 0.62 m/s vs. monocular 0.57m/s; $p<0.01$) were found in all conditions except for the invalid trials in the larger set size condition. 3)There was a significant difference between viewing conditions for the direction of the reaching movement at the time of peak velocity ($p<0.05$). When compared to binocular viewing, participants reaching movements showed a leftward bias during left monocular viewing and a rightward bias during right monocular viewing. The bias was not evident 100 ms after peak velocity suggesting that participants corrected their approach trajectory in the deceleration phase in order to reach the target. There was no significant difference between viewing conditions for the duration of deceleration phase. Conclusion: Binocular vision facilitates the acquisition of relevant information for guiding reaching and grasping movements in cluttered environments. During monocular conditions participants plan their movements more cautiously even in relatively simple environments with highly salient targets. This might be related to increased difficulty in extracting depth information in visually cluttered environments. Monocular input provides sufficient information to engage in online control to correct the initial errors in movement planning.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: Stichting Technologie en Wetenschap (STW), Open Technology Program (OTP) grant 12668.

Title: Repetition: Fundament of adaptation by reinforcement learning?

Authors: *K. VAN DER KOOIJ[1], K. E. OVERVLIET[2], J. B. J. SMEETS[2]
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Abstract: Adaptation to biases between visual and proprioceptive information is generally held to occur by gradually learning from perceived errors that are used to update a model of the visual-proprioceptive mapping. Literature on motor adaptation shows that a second process contributes to the adaptation: reinforcement of successful movements. Can such reinforcement learning also contribute to the learning of perceptual mappings? If so, does such learning depend on repeatedly receiving reward on the same targets? We asked participants to align an unseen hand-held cube with visually projected targets that appeared one by one. After finishing an alignment, participants received reinforcement feedback that introduced a ten-degree rotational bias with an eye-centered origin that participants had to adapt to. That is, the alignment was rewarded if subjects were within a spherical hit-area, ten degrees to the right of the target. The hit-area was gradually reduced by means of a shaping paradigm that kept the reward probability at about 50%. The position of the visual target could be either different on each trial, or at one of four specific positions, presented in a fixed order. We found that adaptation to this rotation, expressed by absolute errors and end point variability, was not improved by repeating a fixed sequence of specific target positions as compared to training with random target positions. Our results suggest that reinforcement learning can contribute to adaptation to visual-proprioceptive biases and does not depend on target repetition.

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Topic: D.05. Visual Sensory-motor Processing

Support: USG Board of Regents

Whitehall Foundation

Alfred P. Sloan Research Fellowship

Title: Posterior parietal cortex predicts upcoming movement during manual interception of moving targets

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Abstract: Although prevalent theories posit that motor control largely relies on forward prediction of sensory consequence, most previous neurophysiological studies have emphasized purely reactive movements toward static targets in which sensory and motor variables are seamlessly linked, making it difficult to distinguish neural activity predicting the resulting movement from that reflecting stimuli location. To elaborate a paradigm in which movement is directed by anticipated sensory outcome rather than current perceived stimuli and reveal underlying neural mechanism, we recorded single-neuron activity from area 7a and area 5d in posterior parietal cortex while monkeys performing a flexible manual interception task. The monkey initiated a trial by positioning a hand at the center of a touch screen for 600-1200 ms. A peripheral target, which appeared and moved along a circular annulus of 30° diameter for 1000 ms, had to be intercepted within this period. Once a peripheral location was touched, the target stopped, and if the angle between target and hand endpoint was less than 15°, the trial was a success and the endpoint was marked for feedback with a red circle (blue if missed). The target moved from one of eight locations spaced at 45°, at angular velocity of 0 (control), 120 or 240°/s clockwise, or 120 or 240°/s counter-clockwise. Eye and hand positions were sampled with an infrared eye-tracker (ISCAN) and electromagnetic tracking system (Polhemus), respectively. In contrast to reactive saccades directed to target location at saccade onset, hand trajectories launched toward final target locations with little online correction, suggesting a feedforward controller to compensate sensorimotor delays. To date, we have recorded 144 task-related cells in area 7a (n=78) and area 5d (n=66) from two monkeys. Tuning curves and preferred directions

were calculated based on firing rate at reach onset under different target motion speeds. For area 7a neurons, movement-directional tuning curves appeared to be invariant ($p > 0.2$), but stimulus-directional tunings were significantly shifted as the target speed ($p < 0.00001$). Partial correlation analysis also demonstrated that pre-movement activity in area 7a was more pronounced to reaching direction than stimulus location. However, this distinction was modest in area 5d, probably because of its closer linkage to movement kinematics in intrinsic coordinates. In such a flexible stimulus-response contingency, PPC explicitly predicts impending movement destination, regardless of the current stimuli, suggesting an intimate role in forward prediction and motor planning.

Disclosures: **Y. Li:** None. **Y. Wang:** None. **H. Cui:** None.

Poster

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DFG Ka 1258/10-1

Title: Delayed pointing in a pure case of ventral pathway damage

Authors: S. CORNELSEN, *M. HIMMELBACH
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Abstract: The so-called perception-action model (PAM) postulates two visual systems. A dorsal pathway from early visual areas to posterior parietal cortex processes information for the control of action whereas a ventral pathway from early visual areas to inferotemporal cortex serves the transformation of visual information for perception. One postulation of the PAM was that the ventral pathway plays an essential role for the guidance of delayed movements to memorized targets. This assertion was only tested in patient DF who not only suffered damage to the ventral pathways but also damage to the dorsal system bilaterally. We examined a stroke patient (HWS) with a unilateral lesion in the ventral pathway who showed lateralized symptoms of visual agnosia. HWS showed accurate immediate reaching towards a visible peripheral target.

However, introducing a delay between stimulus presentation and motor response, HWS was less accurate in his contralesional hemisphere compared to age-matched healthy controls. This observation not only confirms previous findings but also allows for a clear attribution of a deficit in delayed reaching to the ventral stream since HWS suffered from a first-time unilateral stroke resulting in a circumscribed lesion to the occipital-temporal cortex in the right hemisphere.

Disclosures: S. Cornelsen: None. **M. Himmelbach:** None.

Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: NSERC

CFI

Title: Near-hand effect influenced by repetitive transcranial magnetic stimulation applied to AIP

Authors: K. BEBEN, *L. E. BROWN
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Abstract: Placing a hand near a target seems to influence how it is processed. One possible explanation for near-hand effects is that bimodal neuron recruitment contributes to a more robust representation of targets appearing near the hands in comparison to targets far from the hands. Neurophysiological studies have shown that near-hand targets recruit visual-tactile bimodal cells, and that the response of these cells varies with the distance between the target and nearby hand. The purpose of the current study is to determine if the representation of target location for reaching is influenced by the distance between the target and the hand, and if so, whether this effect is ameliorated by the application of rTMS to brain regions known to house bimodal cells. With their right hand, participants reached for targets that appeared either near or far from (1) the participant's invisible resting left hand or (2) a visual cue. Reaching movements were tracked to measure movement timing, and end-point accuracy and precision. Results showed that when the resting hand was present there was a reduction in end-point error variability in comparison to the no-hand condition, a finding consistent with the bimodal recruitment hypothesis. In our second experiment, we used the same behavioural test with participants after they received rTMS

applied to the anterior intraparietal cortex (AIP) or the dorsal premotor cortex (PMd) in the right hemisphere, or underwent a sham procedure. Findings indicated that the near-hand effects recorded in the first experiment were no longer present in participants who received rTMS to AIP. Overall, these results are consistent with the hypothesis that the visual representation of the target is enhanced through the recruitment of multisensory resources when the target appears near but not far from the hand.

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Title: Virtual dissection and comparative connectivity of the superior longitudinal fasciculus in chimpanzees and humans

Authors: *E. E. HECHT[1], D. A. GUTMAN[2], B. A. BRADLEY[5], T. M. PREUSS[3], D. STOUT[4]
[1]Anthrop., Georgia State Univ., Atlanta, GA; [2]Dept. of Biomed. Informatics, Sch. of Med., [3]Yerkes Natl. Primate Res. Center, Div. Neuropharm. & Neurologic Dis., [4]Dept. of Anthrop., Emory Univ., Atlanta, GA; [5]Dept. of Archaeology, Univ. of Exeter, Exeter, United Kingdom

Abstract: Many of the behavioral capacities that distinguish humans from other primates rely on fronto-parietal circuits. The superior longitudinal fasciculus (SLF) is the primary white matter tract connecting lateral frontal with lateral parietal regions; it is distinct from the arcuate fasciculus, which interconnects the frontal and temporal lobes. Here we report a direct, quantitative comparison of SLF connectivity using virtual *in vivo* dissection of the SLF in

chimpanzees and humans. SLF I, the superior-most branch of the SLF, showed no significant differences between humans and chimpanzees, at least using this methodology. SLF II, the middle branch, and SLF III, the inferior-most branch, showed species differences in frontal connectivity. In humans, SLF II showed greater connectivity with ventral premotor cortex and dorsolateral prefrontal cortex. SLF III was right-lateralized in both species, and human SLF III showed greater extension into the anterior inferior frontal gyrus, especially in the right hemisphere. These results are in line with previous studies that have identified relatively recent changes to this network in the human brain, and in particular with research that suggests a unique role for the right anterior inferior frontal gyrus in the evolution of human fronto-parietal network architecture. We suggest that the driving force behind these changes may have been the intertwined selective pressures for spatial attention to observed actions, toolmaking, and social learning, all of which likely depend on integration of spatial, kinematic and sequential information for action perception and control.

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Canadian Foundation for Innovation

Title: The event related potential and microstate analysis of memory guided and visually guided movements

Authors: *G. BINSTED, D. CHENG
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Abstract: Recent examinations of memory-guided reaching using Electroencephalography (EEG) revealed that individuals engaging in memory guided versus visually guided reaches exhibited alterations in neural activity: Both when previewing a target (i.e., target evoked potentials) and when generating a movement to the target (e.g., Krigolson et al., 2012). Notably,

the amplitudes of the potentials were attenuated in memory-guided reaches, largely occurring over the medio-frontal electrodes and attributed to motor planning processes during the target encoding phase - prior to the movement. In the present study, we sought to further examine the involvement of motor planning processes during memory guided movements by varying task difficulty. Specifically, latter components of the waveform (e.g., N2 and P3b) following the visual encoding of an informative cue have been found to scale with index of difficulty (Kourtis et al., 2012). In this experimental procedure, participants performed aiming movements using a stylus on a graphics tablet. At the start of each trial, a target of specified size and amplitude was previewed (250 ms) followed by a 2 s delay in which no target was present. After the delay, an imperative tone was presented informing participants to move to the target. For trials in full vision (FV) the same target reappeared with the tone while for trials without vision (NV) the target did not reappear. Event related potential (ERP) epochs were obtained by time locking EEG segments to target preview, for target encoding, as well as movement start for movement related potentials. The ERP findings showed a general attenuation of neural activity associated with memory-guided reaches as compared to visually-guided ones. This attenuation occurred for both target-evoked and motor related potentials. Moreover, task difficulty-related differences associated with target encoding were found only for visually-guided movements over left central regions, which were contralateral to the acting hand. In addition, time frequency analyses were performed in order to further decipher the frequency band at which the brain activity was predominantly occurring and whether there was related oscillatory activity occurring between brain areas.

Disclosures: **G. Binsted:** None. **D. Cheng:** None.

Poster

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Title: Does moving your limb alter audio-visual temporal order judgments?

Authors: *A. BHATTACHARJEE, T. YAMASHITA, J. DE GROSBOIS, L. TREMBLAY
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Abstract: Goal-directed action yields response-produced feedback that can alter the perception of sensory events. For example, the fusion illusion (perceiving 1 flash when 2 flashes are presented with 1 beep) is less likely to be experienced when reaching towards a visual target (PLoS ONE 5(2010): e8952). This reduction of the fusion illusion during action can be explained by increased visuomotor processing, although such an explanation needs to be tested with other perceptual paradigms. To that end we implemented a temporal order judgment (TOJ) task, which required the participants to judge whether a beep or a flash appeared first. The stimuli were presented at rest (i.e., no-movement condition), and during movements performed either as fast or as accurately as possible. At least the latter condition was expected to favour increased visuomotor processing. In the no-movement condition, 11 stimulus onset asynchronies (SOA) were employed (-320 to +320 ms). During the movements, only 5 SOAs could be implemented (-80 to +80 ms). The movement conditions required the participants to reach towards a visual target, which was aligned with their mid-sagittal axis. The reaching amplitude was 30 cm, parallel to the participant's mediolateral axis. The participants were asked to make a TOJ after each trial. We performed an ANOVA on the likelihood of perceiving the flash first across the 3 conditions and 5 common SOAs. If movements increase visual information processing, the likelihood of perceiving the flash first should be higher with than without movements, at least when the instructions are to be as accurate as possible. The ANOVA on the proportion of flash perceived first data yielded a main effect for SOA as well as a condition by SOA interaction. Post-hoc analysis of the interaction showed that participants were more likely to perceive the flash first in the movement conditions than in the no-movement condition, and that is, when the beep was presented 80 ms before the flash. Using psychometric functions of the no-movement condition data, additional analyses first computed the estimated likelihood of perceiving the flash first at the 0 ms SOA. Next, these estimates were compared to the observed likelihood of perceiving the flash first at the 0 ms SOA for the movement conditions. Such analyses indicated that only movements performed as accurately as possible were more likely to induce the perception that the flash appeared first when the stimuli were presented simultaneously. These results indicate that voluntary action alters audio-visual temporal order judgments and lend further support to the proposal of increased visuomotor processing when reaching to visual targets.

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Wolfson Research Institute for Health and Well- being Early Career Award to CCP

Title: A dissociation between action and perception in patient DF when haptic feedback is constant

Authors: *R. WHITWELL
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Abstract: Patient DF, who developed visual form agnosia following ventral stream damage, configures her hand in flight to match the geometric properties of novel objects when reaching out to pick them up, despite her inability to use these same properties to explicitly differentiate amongst them. For example, DF's anticipatory grip aperture scales to size of objects when she picks them up. Goodale and Milner proposed that her spared grasping is mediated by the visuomotor system housed within the intact areas of her posterior parietal cortex. Alternatively, it has been argued that DF's grip scaling depends entirely on visual feedback, because all previous demonstrations of intact grip-scaling in DF allowed visual feedback throughout her movements. Here, we reject this proposal by showing that DF's grip-scaling is reliable even when visual feedback is removed. A second proposed alternative is that DF's spared grip-scaling is an artifact of her atypical reliance on haptic feedback from the objects which she uses to calibrate egocentric visual cues to the object's surface. This visuo-haptic calibration hypothesis is supported by the fact that DF's grip scaling to visible objects is abolished when there is no corresponding object for her to grasp. There are two problems with this proposal however. First, it is possible that removing haptic feedback shifts the response from 'real' to pantomimed grasps. This is important because both DF and neurologically intact controls are known to perform pantomimed grasps quite differently than they do real ones. Second, because the stimuli used to test DF with and without haptic feedback were not matched for overall size, she could explicitly discriminate one from another. Here, we show that removing haptic feedback altogether does in fact shift the response from 'real' to pantomime grasping in normally-sighted individuals. In an additional experiment, we tested the visuo-haptic calibration hypothesis directly by using stimuli

that DF cannot reliably discriminate amongst. In one of the grasping tasks, DF reached out to grasp blocks of varying visual width but of constant intermediate haptic width. This way, vision and haptic feedback were de-correlated. The results were clear: 1) DF's grip scaling to visual width remained intact even when haptic feedback did not change from trial to trial 2) DF's poor performance when perceptual estimating target width readily dissociated from her good performance when grasping targets of varying width, even when haptic feedback was provided in both tasks. Together, these findings strengthen the proposal that DF's spared grasping is principally driven by the visible geometric properties of the target.

Disclosures: R. Whitwell: None.

Poster

437. Reaching Action

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 437.10/HH1

Topic: D.05. Visual Sensory-motor Processing

Title: Independence of movement planning and movement initiation in a choice reaction time task

Authors: *A. M. HAITH, J. W. KRAKAUER
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Abstract: A typical reaction time (RT) for a goal-directed movement is around 250ms. Signaling delays can account for around 100-150ms of this. Why is the RT usually so much longer? Recent theories have sought to explain the reaction time based on the dynamics of the state of motor cortex during preparation (Churchland et al., J Neurosci, 2006). However, such approaches explain only around 20% of the variability in RT (Afshar et al., Neuron, 2011) and only 30ms of the total RT (Churchland et al., J Neurosci, 2006). Around 100ms remains unaccounted for. We tested subjects' ability to generate accurate reaching movements when preparation time was restricted to be below typical voluntary RTs. Subjects were required to move to one of 8 possible targets equally distributed 8cm from a central start location. We employed a timed-response paradigm (Ghez et al., Exp Brain Res, 1996) in which subjects were trained to initiate movement at a precise time within each trial. We imposed a variety of different preparation times on subjects by manipulating the time at which the target was presented relative to the time of movement initiation. At very low preparation times, subjects failed to hit the target because they were essentially guessing. As preparation time increased, behavior transitioned sharply to

accurate performance. This transition occurred at a preparation time of around 150ms and marked an apparent lower bound on the reaction time which we refer to as the floor RT. Comparing this floor RT to the RTs that were expressed under 'voluntary' conditions (i.e. when subjects were simply instructed to move towards the target as soon as possible after it appeared), we found that subjects' voluntary RTs were, on average, around 100ms greater than their floor RT. Voluntary RTs were also highly variable, with voluntary RT close to the RT floor on some trials, but up to 200ms above it on others. In some instances, subjects even generated voluntary RTs that were lower than their floor RT. On these trials, subjects failed to make an accurate movement. Our findings imply that movement planning, i.e. selection of the appropriate action and preparation of the motor commands, in fact takes very little time. Sluggish initiation and signaling delays account for the bulk of the RT. The high variability in initiation time when compared with the sharpness of the transition at the floor RT, along with the fact that movements are sometimes inadvertently initiated too early suggests that movement initiation and movement preparation are determined by independent, parallel processes rather than being organized serially.

Disclosures: **A.M. Haith:** None. **J.W. Krakauer:** None.

Poster

437. Reaching Action

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Program#/Poster#: 437.11/HH2

Topic: D.17. Voluntary Movements

Support: NSERC Grant 227920-2010

NSERC Postdoctoral Fellowship

Title: Local adaptation of feedback responses and voluntary reaching movements

Authors: *T. CLUFF, S. H. SCOTT
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Abstract: A hallmark of voluntary motor control is the ability to adapt our motor patterns to the physical demands of an ongoing task. Motor adaptation is often studied by quantifying how subjects respond to physical loads applied to their upper limb. Although novel loads initially cause reaching errors, subjects quickly learn to produce accurate movements. This learning

generalizes across movements in the training direction that differ in speed and amplitude, but has limited influence on movements in other directions or locations in the workspace. In parallel, recent studies have investigated how motor adaptation alters feedback responses to mechanical perturbations, highlighting feedback corrections in the long-latency time window (50-105 ms post-perturbation) that rapidly update based on the physical properties of loads applied to the upper limb. These findings highlight an important link between feedback corrections and motor adaptation, leading to the hypothesis that feedback responses should express the same local adaptation patterns observed during voluntary actions. Here we investigate whether feedback responses compensate for novel shoulder loads that were proportional to elbow angular velocity (interaction loads). Subjects (n = 30) first adapted to this load while reaching to three targets in one part of their workspace. We then rotated the shoulder joint and tested for subjects' knowledge of this load while they reached to targets aligned in joint space (i.e., different hand motion, n = 20) or Cartesian space (i.e., different joint motion, n = 10). Feedback responses were measured on random trials by applying perturbations (2 Nm; 10 ms ramp up) that produced elbow motion during the reach. Our results indicate that shoulder muscle responses learn the altered association between elbow motion and shoulder torque. We observed a clear increase in shoulder muscle responses in the long-latency ($p < 0.01$) and voluntary time windows ($p < 0.001$). A critical finding was that adapted feedback responses generalized when subjects reached from different workspace locations to targets requiring identical joint motion patterns (p's < 0.05), but transfer of learning was limited when joint motion patterns differed from the training task ($p > 0.05$). Importantly, reaching errors during learning correlated with the transfer of feedback responses, showing subjects who adapted more to the load displayed greater modulation of their stretch responses across the workspace. We propose that a common learning mechanism governs the adaptation of feedback control and voluntary actions and produces learning that is localized and sensitive to the training conditions.

Disclosures: **T. Cluff:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies.

Poster

437. Reaching Action

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Program#/Poster#: 437.12/HH3

Topic: D.05. Visual Sensory-motor Processing

Support: CIHR MOP 93796

NSERC RGPIN 311680

Title: Visual responses on upper limb muscles during pro- and anti-reach movements implicate the superior colliculus

Authors: *C. GU[1], D. K. WOOD[2], P. L. GRIBBLE[1], T. J. DOHERTY[1], B. D. CORNEIL[1]
[1]Western Univ., London, ON, Canada; [2]Northwestern Univ., Evanstone, IL

Abstract: Previously, short-latency transient recruitment (75-100 ms after visual onset) on the human upper limb muscles has been reported before visually guided reaches. It is thought that this stimulus-locked response (SLR) is mediated via a subcortical tecto-reticulospinal pathway originating in the intermediate layers of the superior colliculus (SC), since the short-latency of the SLR is only slightly longer than the latency of the visual-related response in the SC. To further examine the SLR, we recorded surface and intramuscular EMG activity from both pectoralis major (PM) and supraspinatus (a rotator cuff muscle which stabilizes the shoulder joint) during two reaching tasks in humans. In the first task, subjects reached from a central target towards one of 8 equally spaced possible targets. We observed an SLR on the intramuscular recordings in 7/10 and 4/10 subjects for PM and supraspinatus respectively. Additionally we observed an SLR via surface recordings in PM in 6/10 subjects. In the second task, subjects performed intermixed pro- (towards) and anti- (180° diametrically opposite) reaches to stimuli in the preferred and non-preferred direction of PM, as instructed by a different-colored central target. The same 7 subjects showed a SLR following stimuli presentation in PM's preferred direction, regardless of the direction of the ensuing pro- or anti-reach. The magnitude of the SLR also varied with the task, being: i) smaller before correct anti-reaches versus pro-reaches, ii) larger before incorrect versus correct anti-reaches, and equal before incorrect anti-reaches and pro-reaches, iii) related to the reaction time of correct movement, being larger before short-latency pro-reaches and long-latency correct anti-reaches. Manipulations of background load also revealed that SLRs are more prominent with greater background activity. These features of the SLR show a remarkable resemblance to the visual-related activity in the SC before pro- or anti-saccades, strengthening our contention that the SLR on the upper limb is mediated through a tecto-reticulospinal pathway. If so, the SLR on the upper limb would be a reliable proxy of visual-related SC activity that is accessible in humans.

Disclosures: C. Gu: None. **D.K. Wood:** None. **P.L. Gribble:** None. **T.J. Doherty:** None. **B.D. Corneil:** None.

Poster

437. Reaching Action

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Program#/Poster#: 437.13/HH4

Topic: D.05. Visual Sensory-motor Processing

Support: IRTG 1901 “The brain in action” (DFG)

Title: Reaching for eggs and butter - Integrating spatial reference frames in natural scenes

Authors: *M. KLINGHAMMER[1], L. WALLNER[1], G. BLOHM[2], K. FIEHLER[1]
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Abstract: When interacting with objects in daily life situations, our brain can rely on information represented in two main classes of reference frames; an egocentric (relative to the observer) and an allocentric (relative to objects or the environment) reference frame. So far, most studies investigating how different reference frames are used to guide actions rely on simple and abstract stimuli lacking of ecological relevance. In the present study, we used 3D-rendered scenes of a breakfast table to examine the use of reference frames for reaching in a more naturalistic environment. Subjects freely viewed a scene containing 6 objects on a table (local objects) and 5 objects in the environment (global objects) on a computer screen. After a 2s delay (grey screen) the same scene reappeared for 1s (test scene) but with one local object missing (target). Then the test scene vanished and a grey screen was shown again prompting the subject to perform a reaching movement towards the remembered location of the target. With delay onset, subjects had to fixate on a cross in the center of the screen until the end of the reach. To manipulate the allocentric information derived from the objects in the scenes, all local objects or 1, 3 or 5 global objects in the test scene were shifted horizontally either to the left or to the right. Additionally, the global shifts could be accompanied by a shift of all local objects either in the same or in the opposite direction. We predicted no deviation of reach endpoints from the actual target position on the screen if subjects only used an egocentric reference frame. However, if subjects solely relied on an allocentric reference frame, reach endpoints should deviate from the actual target location by the amount of the objects’ shift. We found reaching errors that lay between our predictions in conditions with local object shifts. Small effects of global object shifts occurred only when they were paired with local objects shifted in the same direction. Effects of global object shifts without a shift of local objects or with a shift of local objects in the opposite direction were negligible. Moreover, in conditions with local object shifts reach endpoints showed higher variability compared to conditions with no local object shift. Our results suggest that allocentric cues substantially contribute to reaching movements, but only if they are task-relevant, i.e. (local) objects in the scene which function as potential reach targets.

Disclosures: **M. Klinghammer:** None. **L. Wallner:** None. **G. Blohm:** None. **K. Fiehler:** None.

Poster

437. Reaching Action

Location: Halls A-C

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Topic: D.05. Visual Sensory-motor Processing

Support: State of Lower Saxony VWZN2563

BMBF Bernstein Center for Computational Neuroscience 01GQ1005C

DFG CRC-889

Title: Dynamic and scalable object-based spatial selectivity in monkey parietal reach region and dorsal premotor cortex

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Abstract: During visually guided reach planning, neurons in monkey parietal reach region (PRR) and dorsal pre-motor cortex (PMd) encode task-relevant spatial information. When reaches are directed towards objects then object shape and size partly determine the relevant space for motor planning. Accordingly, we could show that reach goals are encoded not only relative to the own body or gaze direction (egocentric reference frames), but also relative to the target object (object-centered reference frame) in these areas [1]. Here we test if not only the object position influences the center of reference for spatial selectivity in PRR and PMd, but also if the object size determines the spatial scaling (tuning width) of the neural selectivity. Further, we test if object-centered encoding is temporally stable or specific to specific stages of reach planning [2]. A rhesus monkey was trained to conduct an object-centered reach task. The monkey had to memorize a briefly flashed peripheral visual cue which could occur at one of five locations relative to a memorized extended visual object. After a first delay period (visual memory) the object but not the cue re-occurred. After a second delay (motor planning) the monkey had to reach to the previously cued target location on the object. The first and second object occurrence could be at congruent or incongruent spatial locations. The object had always

the same shape, but could be of the same or different size in both occurrences. Preliminary results suggest that PRR and PMd switch their encoding from predominantly object-centered during the visual memory period to predominantly egocentric during movement planning . We also found that a subset of neurons scaled their spatial selectivity with the size of the object. Our results suggest that neurons in PRR and PMd dynamically adjust their spatial selectivity to the behaviorally relevant spatial space, including spatial parameters of target objects. [1] Taghizadeh B., Gail A.,Object-centered representations in monkey parietal reach region and dorsal premotor cortex. Program No. 162.11. Neuroscience 2013 Abstracts. San Diego, CA: Society for Neuroscience, 2013 Online. [2] BremnerL, Andersen R (2014),Temporal analysis of reference frames in parietal cortex area 5d during reach planning. J Neuroscience 34(15):5273 -5284.

Disclosures: B. Taghizadeh Sarshouri: None. **A. Gail:** None.

Poster

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NIH DP2 NS083037

Title: Complex temporal responses of macaque motor cortex during a novel handle rotation task evoking a range of discreet point-to-point and extended multi-cycle movements

Authors: *B. M. LONDON, M. CHURCHLAND
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Abstract: For over four decades primate motor cortex has been studied predominately using tasks where subjects make directed movements of the arm or wrist to specific target locations. The resulting neural data have formed a rich foundation for formulating hypotheses, which fall into three broad categories: neurons 1) represent limb kinematics, 2) encode muscle activity, or 3) have activity patterns that reflect muscle activity plus related dynamical patterns necessary to generate muscle activity. A natural next step is to ask which of these hypotheses can be expanded to describe neural activity for additional classes of movements. To this end we trained two rhesus monkeys to perform a 'directed volution task.' Monkeys rotated a handle (similar to a bicycle pedal) with their hand to propel themselves through a virtual environment and obtain juice reward at indicated target locations. We evoked 20 distinct movement patterns with differing starting locations, directions, and distances from 0.5 cycles (~450 ms duration) to 7 continuous cycles (~3800 ms duration). Monkeys made movements that began and ended crisply and displayed consistent kinematic profiles across repetitions. We recorded activity from 69 single neurons in motor and premotor cortex and EMG from 9 muscles. Despite simple movement kinematics (nearly sinusoidal modulations of velocity and position), muscle activity was complex, exhibiting departures from purely sinusoidal responses. Neural responses were typically very robust (mean peak firing rate: 71 Hz) and were modulated continuously throughout the movement. Across neurons, responses were highly heterogeneous and exhibited complex features. Neurons often displayed harmonics above cycling frequency or modulation that depended on task epoch (e.g., early versus late in a multi-cycle movement). Many neurons showed preparatory activity, modulating their firing rate prior to movement onset or before finishing a long movement, well before changes in the EMG. Thus, cortical motor regions reflect not only preparation to move, but also preparation to stop. These results indicate that neural responses are considerably more complex than traditional kinematic variables such as velocity. Furthermore, neurons show patterns and features above and beyond what is seen in the EMG. One interpretation is that these extra patterns relate to the neural dynamics that initiate, sustain, and terminate movement. The nature of those dynamics remains to be investigated.

Disclosures: **B.M. London:** None. **M. Churchland:** None.

Poster

437. Reaching Action

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Support: Burroughs Wellcome Foundation

Searle Scholars Program

Sloan Foundation

McKnight Foundation

Grossman Charitable Trust

NIH DP2 NS083037

Title: Neural events when the same movement is initiated in different ways: comparing single-neuron responses during self-generated, cue-driven, and quasi-automatic movements in motor and premotor cortex

Authors: *A. H. LARA, M. M. CHURCHLAND
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Abstract: Given that the same movement can be generated in different ways - thoughtfully, responsively or quasi-automatically - a key question is whether the underlying cortical events are similar across such contexts. Studies using the instructed reach task argue for a key sequence of neural events: cortical preparatory activity leads to movement-related activity, culminating in movement itself. Yet there is evidence that this sequence of cortical events may be superfluous (Perfiliev et al 2010): reaches that intercept moving targets exhibit very short reaction times (RT), suggesting a quasi-automatic, perhaps subcortical substrate. On the other extreme, is the usual sequence of preparatory and movement-related events seen for self-generated movements when there is no time pressure? To study the cortical events underlying reaches in different contexts, we trained two monkeys to perform a center-out reaching task in which the same set of reaches was self-initiated, cue-initiated, or quasi-automatic. In the self-initiated context, monkeys chose when to move based on a tradeoff between a growing juice reward and an internal desire to collect that reward. This context evoked a broad distribution of long-latency movement onset times (616-1124 ms and 670-1202 ms for the two monkeys). In the cue-initiated context, a go cue indicated when movement must be initiated (mean RT = 254 ms and 255 ms). In the quasi-automatic context, a rapidly moving target evoked short-latency intercepting reaches (mean RT = 221 ms and 229 ms). For both monkeys, reach trajectories, speed profiles, and patterns of EMG were similar for the three contexts. Closer investigation of the quasi-automatic reaches revealed that individual RTs ranged as low as 120 ms, and EMG began changing 90 ms after target onset: a latency comparable with neurons in cortical visual areas. To compare the cortical response in these three contexts, we recorded from 110 neurons in primary motor and premotor cortex. When a delay period preceded the go cue, neural events were quite different between the three contexts. E.g., the average correlation between self-initiated and quasi-automatic preparatory response patterns was low: 0.24. However, by the time of movement onset responses were remarkably

similar in all three contexts. E.g., the average correlation between self-initiated and quasi-automatic movement-period response patterns was 0.90, only slightly lower than for the muscles (0.95). Hence, cortex strongly reflects context early, but by the time of movement, neural events are unified across contexts. This argues that cortex cares about context, but that in the end, similar movements are driven by similar cortical events.

Disclosures: A.H. Lara: None. **M.M. Churchland:** None.

Poster

437. Reaching Action

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Topic: D.05. Visual Sensory-motor Processing

Support: Mitsui Sumitomo Insurance Welfare Foundation

Title: Minimal effects of aging on the context-dependent modulation of reflexive correction movements during target reaching

Authors: *K. KADOTA, D. KIMURA, H. KINOSHITA
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Abstract: The ability to compensate for unexpected perturbations is a key function of human motor control. Several studies have demonstrated that this function involves both quick-reflexive and slow-voluntary control processes depending on sensory feedback. We have recently examined how both processes simultaneously contribute to accurate reaching movements. Here, we tested whether aging altered the context-dependent modulation of the gain of the reflexive visuomotor response in order to determine the involvement of higher neural mechanisms in the reflexive response. Sixteen young adults (mean age, 24.6 years; range, 19-32) and fifteen elderly subjects (mean age, 70.1 years; range, 64-79) participated in the study. The participants took part in two experiments that included target-reaching tasks with different task demands. The participants used their right hand to reach for and touch a visual target that was presented in the center of a screen located 0.5 m in front of the participant. After the initiation of the reach motion, the target either shifted (5° right, left, above, or below the screen center) or remained in the center (no-jump trial). In the target-shift trial, the participants were required to correct their reaching trajectory toward the new target location as quickly as possible (protask). In another task, the participants were required to reach an imaginary target that was opposite in direction to

the shifted target (antitask). The participants preformed 2 sets of 96 trials each, for every task. The visual stimulus (target-shift location) was provided in 80% of the trials in random trial order. A reflective marker was placed on the distal interphalangeal joint of the right index finger, and the motion was recorded by a motion-capture system at 500 Hz. The data were filtered, and the velocity and acceleration patterns were calculated. The amplitude of the arm response induced by the visual perturbation was quantified by the difference in the mean hand acceleration between the right and left and between the above and below target-shifted conditions, averaged over a period from 10 ms before to 40 ms after the response onset for the protask. Repeated measures analysis of variance (age group × task) of the response amplitudes revealed that both age and task were significant ($p < 0.01$), but the interaction was not significant. The context-dependent modulation depth of the amplitude was not different between the age groups. This finding suggests that the control center for the modulation of the response is minimally affected by aging; however, the ability for accelerated arm movement in response to visual perturbations declines with age.

Disclosures: **K. Kadota:** None. **D. Kimura:** None. **H. Kinoshita:** None.

Poster

437. Reaching Action

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Program#/Poster#: 437.18/HH9

Topic: D.17. Voluntary Movements

Title: Kinematic characteristics and laterality quotient predict interlimb transfer of sensorimotor adaptation

Authors: ***H. LEFUMAT**[1], J.-L. VERCHER[1], C. MIALL[2], J. COLE[3], L. BRINGOUX[1], C. BOURDIN[1], F. SARLEGNA[1]
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Abstract: Conflicting results have been found on interlimb transfer of reach adaptation. The visual context may be an important factor since in darkness or with indirect visual feedback of the limb, interlimb transfer was observed in contrast to full vision. Cognitive factors and credit assignment (i.e. the source of errors) have also been suggested to play a critical role in interlimb transfer. We tested the influence of sensory feedback and conscious awareness on interlimb

transfer. We hypothesized that without visual feedback of the arm, after-effects may be found on the non-exposed non-dominant arm (NDA) after adapting with the dominant arm (DA) to a novel force field, but not with direct vision that may enhance the association of the trajectory errors to internal causes. Since proprioception has been shown to be important for updating the internal model of limb dynamics, we also tested proprioceptively-deafferented subjects and hypothesized that they would not be able to transfer. Subjects reached for visual targets in a rotating platform producing a velocity-dependant force field. Ten right-handed young adults had no visual, only proprioceptive feedback of hand movement (P group), while 10 other right-handed young adults had direct vision of the limb (VP group) as well as 2 deafferented subjects (GL: right- and IW: left-handed, V group). There were 4 experimental phases: pre-rotation (DA/NDA), per-rotation (DA), post-rotation (NDA/DA) and lastly a questionnaire about credit assignment issues. In all 22 subjects, reaching arm movements were initially perturbed but kinematic parameters rapidly returned to baseline. On average in each group, we observed interlimb transfer to the untrained NDA, as reflected by the significant difference between initial movement direction in baseline and the first trial of post-rotation. The questionnaire analysis showed that conscious awareness of the error, or conscious attribution of trajectory errors to internal or external causes, did not significantly influence interlimb transfer. Thus, we found no evidence that visual or proprioceptive feedback, or conscious mechanisms determine interlimb transfer. We noticed a substantial inter-subject variability in transfer and developed a discriminant model which correctly predicted the presence or not of interlimb transfer for 95% of the subjects based on 3 variables: variability of initial movement direction and peak velocity of the RH during the adaptation phase, and laterality quotient. Greater variability, peak velocity and laterality quotient predicted interlimb transfer of force-field adaptation.

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Poster

437. Reaching Action

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 437.19/HH10

Topic: D.17. Voluntary Movements

Support: NSERC

Title: The human motor system adapts reaching movements for both task-relevant and task-irrelevant forces

Authors: *J. G. CASHABACK[1], H. R. MCGREGOR[1,2], P. L. GRIBBLE[1,3]
[1]Brain and Mind Institute, Dept. of Psychology, [2]Grad. Program in Neurosci., [3]Dept. of Physiol. and Pharmacol., Western Univ., London, ON, Canada

Abstract: The minimum intervention principle and the uncontrolled manifold hypothesis state that we only respond to environmental forces and sensorimotor noise if they affect task success. That is, our nervous system compensates for task-relevant forces and noise, but ignores them if they are task-irrelevant. Such a flexible response is theoretically possible by exploiting muscle, joint, or workspace redundancy (e.g., large targets). While there is strong evidence that we capitalize on joint redundancy and conflicting evidence that we utilize muscle redundancy, limited research exists on whether we are able to exploit workspace redundancy. Here, we test the hypothesis that we respond to task-relevant forces and ignore task-irrelevant forces in a reaching task involving targets of different sizes. Three groups of participants performed reaching movements in a horizontal plane while grasping the handle of a robot manipulandum. Participants started from a home position, then moved rightwards through two small via point targets and finally stopped within a large, curved, rectangular target. The task-irrelevant (TI) group received a counterclockwise force that acted parallel to the surface of the large target. Crucially, this force had no affect on task success as it simply pushed the participant's hand into another area of the large target. The task-relevant (TR) group received a unidirectional force that pushed them away from the second via point. The NULL group did not receive any perturbing forces. Interestingly, results show that the TI group significantly modified their hand trajectories compared to the NULL group, immediately before being perturbed by the task-irrelevant, counterclockwise force ($p < 0.05$). As expected, the TR group modified their movements prior to receiving the unidirectional force ($p < 0.05$), allowing them to successfully pass through the second via point target. In summary, participants altered their movements prior to being perturbed—even when the perturbing force had no bearing on task success. Our results are not consistent with the minimum intervention principle and uncontrolled manifold hypothesis. Future work will explore the underlying motives for altering kinematic behavior prior to receiving task-irrelevant perturbations.

Disclosures: J.G. Cashaback: None. **H.R. McGregor:** None. **P.L. Gribble:** None.

Poster

437. Reaching Action

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Program#/Poster#: 437.20/HH11

Topic: D.17. Voluntary Movements

Support: CREST, JST EH

Title: The cingulate motor area of monkeys is involved in specification and initiation of reaching movement

Authors: *T. YAMAGATA[1], L. TREMBLAY[2], E. HOSHI[1,3]
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Abstract: The cingulate motor area (CMA) is thought to be involved in sensorimotor control and internal generation of movement. To reveal the neural mechanisms, we developed a behavioral task for monkeys in which it was required to execute reaching movement in an externally triggered or a self-initiating manner. A trial commenced when a colored fixation spot instructing the initiation mode (green, externally triggered mode; magenta, internally induced mode) and four peripheral potential targets (gray) appeared on a screen that was installed in front of the monkey. If the monkey continued to gaze at the fixation spot, the color of one target turned cyan to indicate a correct target position. In the externally triggered mode, after a waiting period, the trigger sound was delivered to inform the monkeys to initiate reaching movement to the target. In the self-initiating mode, after a prescribed time period, the monkeys initiated reaching movement without any trigger signals. While monkeys performed the task, we recorded neurons in the CMA. By comparing the neuronal representations in the two modes, we obtained three major findings. (1) After the correct target presentation, CMA neurons began to reflect action. (2) During movement preparation, CMA neurons reflected the initiation mode, while the information on action was maintained. (3) After movement initiation, the selectivity for initiation mode decreased, whereas the selectivity for action increased. These results indicate that the CMA of monkeys is involved in both specification and initiation of action.

Disclosures: T. Yamagata: None. **E. Hoshi:** None. **L. Tremblay:** None.

Poster

437. Reaching Action

Location: Halls A-C

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Program#/Poster#: 437.21/HH12

Topic: D.18. Brain-Machine Interface

Support: UL1 TR000448, sub-award TL1 TR000449

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R01 HD061117-05A2

Title: Prediction of directional kinematics from 3D reaching movements of the contralateral and ipsilateral limb using electrocorticography

Authors: *D. T. BUNDY[1], N. SZRAMA[1], M. PAHWA[1], C. HACKER[1], M. SHARMA[1], E. C. LEUTHARDT[2]
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Abstract: Electrocorticography (ECoG) has emerged as a signal platform for potential brain-computer interface (BCI) applications in motor-impaired patients. Traditionally, research studies examining neural representations of motor intention for BCI applications have focused on the cortex contralateral to a moving limb. Although this would be ideal for clinical populations with an intact cortex, these signals would likely be altered or lost after a hemispheric stroke. Furthermore, while previous studies have demonstrated a unique neural physiology related to movements of the ipsilateral limb, the ability to decode directional kinematics is uncertain. This study examined whether ECoG signals can be used to decode kinematics of 3D reaching movements of both the ipsilateral and contralateral limbs for potential BCI applications. Five intractable epilepsy patients were implanted with subdural ECoG grids. ECoG signals and 3D hand position were simultaneously recorded while patients performed 3D center-out reaching movements. During one session, reaches were made with the arm contralateral to the electrode grid, and during a second session, reaches were made with the arm ipsilateral to the electrodes. Signals were examined to identify signal characteristics related to kinematic information and a regularized common spatial patterns algorithm was used to develop frequency band-specific spatial filters for decoding of kinematic information. Machine learning techniques were then used to decode kinematic information about reaching movements from ECoG signals. ECoG signals over sensorimotor, premotor, and frontal cortices had significant spectral power changes during the planning and execution of movements of both the contralateral and ipsilateral limbs relative to baseline. Additionally, the data demonstrates that cortical activity is characterized by unique similarities and differences in the relationship between neural activity and movement kinematics of the contralateral and ipsilateral limb based upon cortical location and frequency band. Furthermore, through the calculation of unique band-specific spatial filters, we demonstrate the ability to decode information about directional kinematics of both arms from a single cortical hemisphere. These results demonstrate the presence of neural representations of contralateral and ipsilateral limb kinematics in human ECoG signals. Furthermore, the

demonstration of the ability to decode kinematic information from ECoG signals demonstrates the potential for these findings to be extended to the future development of a BCI system for use in patient populations after hemispheric stroke.

Disclosures: **D.T. Bundy:** F. Consulting Fees (e.g., advisory boards); Neurolutions. **N. Szrama:** None. **M. Pahwa:** None. **C. Hacker:** None. **M. Sharma:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions.

Poster

437. Reaching Action

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Topic: D.05. Visual Sensory-motor Processing

Support: NSF Grant IOS-0746398

Oak Ridge Associated Universities Postdoctoral Fellowship Program

Title: Interaction of sensory and motor noise during reaching: A simulation study

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Abstract: Reaching movements are inherently variable, partly due to noise in sensorimotor processing associated with the planning and execution of limb movements. Execution noise is typically used to describe variability associated with generating motor output, manifesting largely as variability along the terminal movement vector; planning related noise depends largely on the anisotropic nature of sensory uncertainty. Strong behavioral evidence suggests that the brain coordinates sensorimotor processes to minimize variability, which has fostered the belief that the brain exploits the characteristics of sensory and motor noise to optimize reaching performance. To evaluate this claim, we developed a feedback control model augmented with a Kalman filter to assess the influence of anisotropic sensory uncertainty (planning noise) on endpoint control of reaching. Simulations of 2D movements were performed with distinct characteristics of feedback variability: Zero noise, isotropically distributed noise, and noise representative of known visual and proprioceptive feedback uncertainty. In addition, the model

was developed to integrate multiple feedback inputs in order to evaluate the effect of multimodal sensory feedback on endpoint variability. Simulated reaching performance with isotropic feedback noise yielded patterns of variable error similar to those resulting from pure execution noise (zero feedback noise). On the other hand, movements with anisotropic feedback noise produced endpoint variability which more closely resembled patterns of feedback uncertainty, particularly with respect to the aspect ratios of error distributions. Multimodal simulations also produced distinct patterns of endpoint variability, reflecting unique contributions of feedback and execution noise depending on the direction of movement. In all cases, total error volume was consistent with a 'near optimal' combination of planning and execution noise, suggesting that endpoint variability under optimal sensorimotor control arises from the interaction of execution noise with anisotropic sensory feedback. Finally, movements were also simulated with variable onset/offset of sensory feedback. The influence of execution noise was more apparent in these delayed feedback simulations. However, the tendency for delayed visual feedback to reduce movement variability and increase the aspect ratio of endpoint distributions was much greater than for proprioceptive feedback, suggesting the more reliable visual signal more rapidly influenced estimates of limb position during movement.

Disclosures: G.A. Apker: None. **C.A. Buneo:** None.

Poster

437. Reaching Action

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01 NS079664

Title: Location then object representations sequentially predominate in the widely distributed activation of the primary motor cortex during reach to grasp

Authors: *A. G. ROUSE, A. T. ROUSSIN, M. H. SCHIEBER
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Abstract: The primary motor cortex (M1) upper extremity representation of macaques may be viewed as having a central core of distal representation surrounded by a "horseshoe" of proximal representation. Neurons in the core and horseshoe each are considered to represent particular features of muscle activity and/or related movement kinematics and dynamics. To the extent that

reach and grasp proceed in parallel, this model would predict that activation in the horseshoe related to reach location and activation in the core related to grasp shape would proceed in parallel. We recorded spiking activity from microelectrode arrays implanted in M1 as two monkeys (Macaca mulatta) reached and grasped one of four objects in up to eight different locations. Mean firing rates and depth of modulation of unit tuning across trial types peaked at two time points. The first began before movement onset; the second, larger peak preceded object contact. Time-resolved two-way analysis of variance of individual single- and multi-unit recordings revealed that the first peak was dominated by location effects, whereas the second peak was dominated by object effects. We therefore examined the spatial distribution of location and object effects as a function of time. Prior to movement onset, location effects were found in spikes recorded throughout the M1 upper extremity representation. Object effects appeared primarily after movement was underway, and grew concurrently in spikes recorded throughout most of the upper extremity representation. Although a slightly higher percentage of spikes in the central core were object-related while a slightly higher percentage in the medial and lateral fringes were location-related, the temporal shift predominated, with many units switching from location to object representation over the time course of trials. Our observations suggest that a stationary model characterized by i) a core of distal representation surrounded by a horseshoe of proximal representation, and ii) fixed tuning of individual spikes to particular features of muscle activation and/or motion kinematics/dynamics, may be an inadequate characterization of M1 during reach to grasp. Our observations suggest that widely distributed but low amplitude activity in the M1 upper extremity representation initially represents transport of the extremity to an appropriate location. Subsequent activity of increasing amplitude--often in neurons previously tuned to location--thereafter represents the shaping of the extremity to grasp the object. Neuroprosthetic applications that decode M1 activity might be improved by accounting for this temporal transition in widely distributed activation.

Disclosures: **A.G. Rouse:** None. **A.T. Roussin:** None. **M.H. Schieber:** None.

Poster

437. Reaching Action

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Topic: D.17. Voluntary Movements

Support: NIH Grant 5R01NS050256

Title: Short time-scale stability of directional tuning in motor cortex measured using maximum likelihood estimation

Authors: ***S. B. SUWAY**[1], A. J. C. MCMORLAND[3], G. W. FRASER[2], J.-W. SOHN[2], S. M. CHASE[5], Z. LIU[6], M. VELLISTE[2], R. E. KASS[6], A. B. SCHWARTZ[4]
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Abstract: The activity of motor cortical neurons is often related to hand kinematic parameters using linear regression techniques. This yields robust model predictions when firing rates are averaged over entire movements and regressed against the cosine of reach direction, with peak firing predicted for a single direction called the "preferred direction" (PD). However, when regressed repeatedly over several shorter windows of time, the resultant models often produce strikingly different estimates of PD. We hypothesized that noisy estimates of firing rate might result in poor model fit, especially for low spike counts. While the standard cosine model imposes a fixed shape to the tuning function, a neuron's "preferred direction" is, in the broadest sense, the movement direction that elicits maximal firing. To minimize assumptions about an underlying model, we adopted this latter definition as the basis for a maximum likelihood estimation (MLE) approach to measuring PD. We recorded single neurons in primary motor cortex and optically tracked hand position while monkeys (two males, Macaca mulatta) performed three-dimensional point-to-point reaching in virtual reality. Single units were isolated using offline spike sorting, and spike counts were binned in ~50 ms time windows. We then analyzed the instantaneous movement directions associated with just the highest 15-20% of firing rates independently for each window, and calculated the probability of reach direction given high firing rate. We regressed our neural data against whole-trial kinematics (direction, position, and speed), assuming that PDs are fixed in time. We then simulated Poisson-distributed spike counts for each sample using the model predictions, and repeated our MLE analysis on these simulated neurons. For the vast majority of real neurons, we find that the amount of excursion observed in MLE of PD is highly comparable to what we predict from simulated neurons with static preferred directions. Stability in the MLE of PD is most prominent during samples in the middle of the reach, when firing rates tend to be highest. We suggest that observed changes in PD when assessed on short time-scales are due to noise and poor model fit, rather than a change in the neuron's tuning characteristics.

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Poster

438. Eye Movements: Cortex

Location: Halls A-C

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Program#/Poster#: 438.01/HH16

Topic: D.06. Eye Movements

Support: CIHR

Title: A novel multi-barrelled glass coated silver-plated tungsten wire tetrode for combined extracellular recording and microiontophoresis in the primate brain

Authors: *S. VIJAYRAGHAVAN, A. J. MAJOR, S. EVERLING
Western Univ., London, ON, Canada

Abstract: Microiontophoresis combined with extracellular recording has been used extensively to study the pharmacology of neurons and important insights have been gained about the effects of neuromodulation of neurons engaged in active behaviour. Single-unit recordings with concomitant application of neuroactive agents have traditionally been carried out with glass micropipettes in various configurations, including glass-coated tungsten or carbon fiber microwires in a multibarrel array. These electrodes have the advantage of inexpensive fabrication and design flexibility that is required for recordings in the large primate brain. A drawback of single wire iontophoretic physiology is the variable effect on spike shape of application of pharmacological agents. Further, the inability to clearly distinguish multiple units at a recording site limits the potential of the methodology to study local circuit interactions. Spike amplitudes have been found to be susceptible to overall firing rate, firing mode, temperature, and factors that affect the resting membrane potential. These factors can be exacerbated by pharmacological manipulation. Stratton et al (PLoS ONE, 7(6), 2012) examined the effects of firing rate changes induced by glutamate and GABA iontophoresis on spike variability, and found that spike amplitude and spike duration vary with changes in firing rate. Microwire tetrodes have been used typically to enhance unit isolation. These bundles are additionally plated with gold or platinum to reduce their impedances and associated Johnson noise, thereby improving unit yield. Likewise, silver-coated iontophoretic electrodes show reduced impedances across the frequency spectrum. Tetrodes have the potential advantage of maintaining unit isolation notwithstanding factors inducing non-stationarities in waveforms. In this study, we describe a 7-barrelled glass tetrode, with 4 silver-coated 12-micron tungsten microwires tapered to <1 micron and 3 iontophoretic barrels. Tip sizes can be brought down to 30-100 microns. Silver coating reduced impedances 5-10 fold in the DC range and electrodes with typical 1 kHz impedances of 80-200 kOhms could record units with high signal-noise

ratios. These electrodes have the ability to eject neuropharmacological agents with low noise recordings and reliable unit isolation, which will facilitate the study of the pharmacology of neuronal microcircuits.

Disclosures: S. Vijayraghavan: None. **A.J. Major:** None. **S. Everling:** None.

Poster

438. Eye Movements: Cortex

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: D.06. Eye Movements

Support: CIHR

Title: Cholinergic modulation of prefrontal cortical activity subserving mnemonic representation of rule-guided behaviour

Authors: *A. J. MAJOR, S. VIJAYRAGHAVAN, S. EVERLING
Western Univ., London, ON, Canada

Abstract: The ascending telencephalic cholinergic neuromodulatory system has an important role in arousal, and has been implicated in several higher-order cognitive functions, including attention, episodic memory, and working memory (WM). The prefrontal cortex (PFC) has been intimately associated with these higher-order functions. Dorsolateral PFC (DLPFC) function, specifically, has been found to be essential to performance in tasks requiring attention, WM, and rule-guided flexible behaviour. Pathology of the basal forebrain cholinergic system has been implicated in Alzheimer's Disease and Schizophrenia. Pharmacological investigations of cholinergic actions have been studied in attentional tasks in striate and extra-striate cortices and PFC during visuospatial WM, and in inferotemporal cortex and PFC during episodic and recognition memory. Cholinergic denervation of PFC selectively affects spatial WM in monkeys, while administration of muscarinic cholinergic antagonist scopolamine in rat medial PFC leads to dissociable effects on spatial WM and attention, and elevated responses in the parahippocampal gyrus during recognition memory encoding. Cholinergic involvement in mnemonic activity for rule-contingent behavioural processing has not been well-studied hitherto. Performance in the antisaccade task, which combines spatial perceptual processing, abstract-rule memory, and prepotent response inhibition is dependent on DLPFC integrity and is impaired in neuropsychiatric disorders. We investigated the effects of scopolamine and cholinergic agonist

carbachol microiontophoretically applied on DLPFC neurons in monkeys performing a rule-memory antisaccade task. Scopolamine inhibited a majority (62%) of 78 DLPFC units tested, including 15 units that displayed preferential activity for one rule. Activity was augmented in 13/78 units. Suppression of rule-memory activity reduced rule-selectivity of the units. Carbachol application, in contrast, had more mixed effects. Cholinergic activation through carbachol inhibited 20/34 units tested, while 6/34 showed enhancement. Consistent with other findings, our results support a role for the muscarinic cholinergic system in PFC cognitive functions, specifically in rule maintenance for flexible behaviour.

Disclosures: A.J. Major: None. **S. Vijayraghavan:** None. **S. Everling:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: CIHR

Title: Ketamine-induced changes in the signal and noise of prefrontal neurons during a working memory task in monkeys

Authors: *L. MA[1,3], K. SKOBLENICK[4], J. K. SEAMANS[2], S. EVERLING[3,4,5,6]
[1]Grad. Program in Neurosci., [2]Brain Res. Center, Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [3]Physiol. and Pharmacol., [4]Anat. and Cell Biol., [5]Psychology, [6]Robarts Res. Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Acute injection of subanesthetic dose of Ketamine in non-human primate is an effective model for creating schizophrenia-like symptoms, such as deficits in working memory. We trained two rhesus monkeys to perform a rule-based working memory task involving pro- and anti-saccades, and recorded neuronal ensembles from the dorsolateral prefrontal cortex both before and after Ketamine injection. Previously we reported that Ketamine injection negatively impacted the maintenance and the application of the correct task rule, and increased the reaction times of both pro- and anti-saccades. We hypothesized that a reduction in neural signal-to-noise ratio (S/N) during the delay may underlie the deterioration of performance induced by Ketamine. The 'signal' is defined as the difference between the averaged neural responses to the two task rules during the delay period, and the 'noise' is defined as trial-to-trial variation in delay activity.

On the ensemble level, we found that Ketamine injection reduced S/N during the delay period, even when only correct response trials were considered. Furthermore, this reduction in S/N was caused by an increase in the level of noise in ensemble activities while the signal remained constant. On the single neuron level, only broad-spiking neurons (BSNs, putative pyramidal neurons), but not narrow-spiking neurons (NSNs, putative fast-spiking interneurons), showed significant reduction in S/N, and this occurred due to an increase in noise level without any change in the strength of the signal. Given that the effect of Ketamine on individual BSNs matches the ensemble effect, we also analyzed the S/N of ensembles consisting purely of BSNs. Indeed, the S/N before and after Ketamine injection in BSN-only ensembles were nearly identical to those of mixed ensembles. Meanwhile, Ketamine also has its unique effect on NSNs: it significantly altered the temporal structure of NSN activities, reflected by a significant increase in the coefficient of variance (CV) during the delay in both pro- and anti-saccade trials_a phenomenon not observed in the BSNs. Together the results suggest that the effects of Ketamine differ in different types of neurons, which in turn differentially contribute to its effect on ensemble coding of working memory information.

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Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Title: Rebound in latencies distribution reveals cortical involvement in saccadic inhibition

Authors: *P. POUGET[1], P. DAYE[2], S. RIVAUD-PÉCHOUX[2], N. WATTIEZ[2], B. GAYMARD[2]
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Abstract: Presentation of visual distractors away from the target and shortly after its onset holds off the initiation of a large proportion of saccades but has little effect on landing positions (Reingold and Stampe, 2000). Behaviorally, this delay generates a “dip” followed by a “rebound” in the latency distribution. Physiologically, the saccadic inhibition is assumed to be the outcome of a competition between the target and the distractors in the visuomotor maps of the superior colliculus (SC) without any cortical involvement. This assumption was drawn from

the sole analysis of the dip characteristics. To disentangle between a native collicular mechanism and a cortico-colicular mechanism, we performed a new series of experiments while manipulating the target saliency and we analyzed how it modulates the rebound in the latency distribution. 15 subjects performed several series of pro- and antisaccades. Each trial started with a 500 ms central fixation cue (red=pro-saccade, blue=anti-saccade). Then, the target appeared randomly either leftward or rightward (random amplitude [4, 8, 12]°) during 90 ms. To modulate the target saliency, four mask conditions and two color conditions were mixed and kept constant during a block of trials. Under the first mask condition ("no mask"), only the target was presented on the screen. For the second, a structural mask covered the whole screen ("full mask"). For the third and fourth mask conditions, the mask covered half of the screen either on the same ("ipsi") or the opposite ("contra) side of the target. Finally, to maintain the target saliency independently of the mask condition, the color of the target either matched the mask color (target invisible) or not (target pop out). 21534 pro-saccades and 21174 anti-saccades trials were analyzed. When the mask was presented contralaterally to the target, the results showed a fast and strong recovery of the delayed saccades. When the mask and the target were on the same side, the rebound duration was longer and its amplitude smaller. It is currently accepted that the outcome of target saliency involved cortical mechanisms. Therefore, our results raise a question: how can we reject a cortical modulation of the saccadic inhibition if the rebound is modulated by the target saliency? References: Reingold EM, Stampe DM (2000) Saccadic inhibition and gaze contingent research paradigms. Elsevier, Amsterdam.

Disclosures: P. Pouget: None. **P. Daye:** None. **S. Rivaud-Péchoux:** None. **N. Wattiez:** None. **B. Gaymard:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: NIH grant EY019663

Title: An intrinsic inhibitory rostrally-directed intralaminar pathway in the rodent superior colliculus

Authors: *P. BAYGUINOV[1], M. B. JACKSON[1], M. A. BASSO[2]
[1]Neurosci., Univ. of Wisconsin, Madison, WI; [2]Psychiatry and Biobehavioral Sci., Univ. of California, Los Angeles, CA

Abstract: The superior colliculus (SC) is a midbrain structure that functions in the detection of spatial visual input and the generation of saccadic eye movement. Work in awake non-human primates has established the behavioral function of the SC, and work in brain slices from rodents has provided insight into how synaptic circuits of the SC may generate this behavior. Using synthetic dye voltage imaging to explore population dynamics, and whole cell patch clamp recording to measure synaptic responses, we have explored the spatiotemporal dynamics of intrinsic SC circuits along the rostro-caudal axis of rat sagittal SC slices. Voltage imaging revealed that stimulation in either the superficial sensory or intermediate premotor layers activated superficial layers asymmetrically, with greater spread caudally than rostrally (provided that stimulation was applied more than ~1 mm from the caudal end). Application of the $GABA_A$ receptor antagonist SR95531 (5 μM) reduced the skew in the spread of depolarization, making the superficial layer responses more symmetrical around the site of stimulation. $GABA_B$ receptor antagonists had no effect on the spatial distribution of responses. Whole cell patch clamp recordings, and subsequent morphological analysis of cells receiving inputs from sites either more rostral or more caudal, revealed a greater percentage of cells at sites rostral to a stimulus received inhibitory inputs, compared with cells caudal to the stimulus. Dual-site stimulation experiments, with temporal offsets, showed that rostrally-directed inhibition could impede the propagation of responses to subsequent stimuli within the superficial layers. Together, these data revealed a novel intralaminar $GABA_A$ receptor-mediated inhibitory pathway within the SC that limits the spread of responses in the rostral direction. This pathway can serve to inhibit small amplitude saccades to sites less distant than a selected target, as occurs in the behavioral phenomenon of corrective saccades. Supported by NIH grant EY019663.

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Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: UGA OVPR and BIRC

Title: Modulation of the neural correlates of saccade performance using task switching and trial type probability in an event-related fMRI paradigm

Authors: *J. E. PIERCE, J. B. MCCARDEL, J. S. COPPIANO, A. L. RODRIGUE, D. J. SCHAEFFER, S. ARKIN, J. E. MCDOWELL
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Abstract: Task performance changes based on whether the current trial type differs from the preceding trial type. To investigate the neural bases of these task switching processes, two types of saccadic eye movement tasks were presented in several runs of interleaved trials. A prosaccade is a basic, reflex-like glance towards a newly appearing stimulus, while an antisaccade is a more complex response requiring a glance to the mirror image location of the stimulus that utilizes greater levels of cognitive control. Switching between these two saccade types requires remapping of stimulus-response pairings, as the same peripheral stimulus is used to cue the eye movement in both trial types. In the current study, the relative proportion of pro- to antisaccades was manipulated across three runs to investigate the effects of trial type probability on participants' task set modulation. Prosaccade performance is supported by well-documented cortical circuitry that includes visual cortex, posterior parietal cortex, and frontal and supplementary eye fields. Greater activation in these regions, as well as additional regions including prefrontal cortex, may be recruited to support antisaccade performance. The level of activation in saccadic circuitry, as indexed by the BOLD fMRI signal, is expected to differ based on the context of the previous trial type or the overall trial type probability within the run. Twenty-six healthy undergraduate students completed three event-related fMRI runs. The runs differed in the relative proportion of pro- and antisaccade trials: 25%, 50%, and 75% prosaccades. A 3x2x2 (Probability Run x Trial Type x Task Switching (repeated/switched)) within-subjects ANOVA was conducted. For prosaccades, five clusters showed significantly different activation between repeated and switched trials. Greater activation was seen during repeated prosaccades in: bilateral visual cortex, left basal ganglia/superior temporal gyrus, and bilateral post-central gyrus. Greater activation was observed during switched prosaccades in: right posterior parietal and dorsolateral prefrontal cortex, particularly for the 25% prosaccade run. This pattern of results suggests that basic motor/sensory areas are involved to a greater degree when the same task set is repeatedly invoked; however, regions of the dorsal attention network are activated when switching from a complex response (antisaccade) to a more reflexive response (prosaccade), especially when the probability of a prosaccade trial is low. Task switching processes may have been recruited on these trials to reconfigure the active saccade task set and appropriate motor response based on given instructions.

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Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: CIHR MOP 93796

NSERC CREATE

Title: Impairment, but not abolishment, of express saccade generation following large and reversible cryogenic inactivation of frontal eye fields (FEF)

Authors: *S. DASH[1], S. G. LOMBER[2], B. D. CORNEIL[3,2]
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Abstract: The introduction of a temporal gap between the disappearance of the fixation point and appearance of the saccade target (gap saccade paradigm) promotes the generation of short latency "express" saccades (< 100ms). The role of the FEF in the generation of express saccades remains unclear. On one hand, monkeys with permanent unilateral lesions of the FEF are still capable of generating contralesional express saccades after a period of recovery, suggesting that the integrity of the FEF is not essential for express saccades. On the other hand, many saccade-related FEF neurons increase their activity during the 200 ms gap before express saccades. Such buildup or fixation-disengagement activity is also seen in the SC before express saccades, suggesting that the FEF may be essential for express saccade generation in the intact animal. The effect of FEF inactivation, unconfounded by recovery, on express saccade generation is not known. In the present study we set out to study the acute effect of large yet reversible FEF inactivation on the generation of express saccades. To do this, we performed reversible unilateral and bilateral cryogenic inactivation (cooling) of FEF using cryoloops implanted in the arcuate sulcus in 2 rhesus monkeys during a 200 ms gap saccade paradigm. Targets were randomly placed at 6 potential locations along the horizontal axis (3 in each direction). In a typical session, we collected data before cooling, then during unilateral FEF cooling, then during bilateral cooling, and finally after rewarming. This sequence allowed us to compare the effects of both unilateral and bilateral inactivation. Surprisingly, while unilateral FEF inactivation increased contralateral saccade latencies in both animals, both animals retained the ability to occasionally generate contralateral express saccades. In one monkey, such contralesional deficits recovered

upon bilateral FEF inactivation in a pattern reminiscent of a Sprague-like effect, with the animal capable of producing express saccades in either direction. In the other monkey, bilateral inactivation increased saccadic latencies and decreased (but did not abolish) the probability of express saccades in both directions. Together, these results demonstrate that the FEF has a contributing, but not essential, role in express saccade generation.

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Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Title: Neural correlates of spatially and temporally predictive saccades

Authors: *B. J. CHANG, D. C. BRIEN, B. C. COE, D. P. MUNOZ
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Abstract: Prediction is the process of using information from either the past or present to guide future behaviour. It is needed to compensate for neural delays between a sensory input and an appropriate motor output. We designed an eye movement task to clarify the behavioural control and neural correlates that are involved in both temporal and spatial prediction. A task involving temporally and spatially predictive and non-predictive saccades was employed in an MRI in which four conditions were tested: spatially/temporally predictive (STP), temporally predictive/spatially non-predictive (TP), spatially predictive/ temporally non-predictive (SP), and spatially/temporally non-predictive (NON). Data from 24 normal human subjects (mean age = 22.4 yrs) showed distinct behavioural differences between conditions. All subjects elicited primarily predictive saccades (saccadic reaction time: SRT < 100ms) in the STP condition. The NON condition elicited primarily reactive saccades (SRT > 100ms). The average SRT of the SP condition fell between the average of the STP and NON conditions, and no significant differences in SRT were observed between the TP and the NON conditions. Analysis of the functional imaging data identified regions of interest with activations that correlated to the predictive conditions. Contrasts of predictive conditions isolating both spatially and temporally predictive areas showed activation of the parietal eye fields (PEF), insular cortex, and medial prefrontal cortex (MPFC) which may play a role in the control of predictive saccades. Contrasts that isolated spatially predictive areas also showed activation of the PEF, insular cortex, and

MPFC while contrasts that isolated temporally predictive areas showed select activation of the cerebellum which may serve as the internal clock that drives the regular rhythmic behaviour observed for the temporal aspect of predictive saccades. Surprisingly, activation or frontal areas responsible for saccadic control such as the Frontal and Supplementary Eye fields were equal among all conditions. The behavioural differences validated the activity of the contrasts to isolate brain areas that are correlated with both spatial and temporal prediction. The results from these contrasts indicated that brain activation in the STP/SP and TP conditions reflect predictive responses to visual stimuli and motor-timed responses, respectively. This suggests that utilizing a predictive saccade task is a valuable tool for simultaneously testing both spatial and temporal prediction that involves fast internally-guided responses.

Disclosures: **B.J. Chang:** None. **D.C. Brien:** None. **B.C. Coe:** None. **D.P. Munoz:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

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Title: The phase of ongoing EEG oscillations predicts the amplitude of peri-saccadic mislocalization

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Abstract: Updating processes in response to body, head and eye movements, such as saccadic remapping, are essential for the maintenance of a spatial representation of the world around us. It has been proposed that, rather than continually update a full spatiotopic map, only the location of a few attended objects need be remapped, leading to the idea of 'attention pointers'. At the same time, there is mounting evidence linking attention to oscillatory neuronal processes. We therefore hypothesised that remapping processes should themselves show oscillatory characteristics, inherited from underlying attentional processes. In order to test this, we carried out a combined psychophysics and EEG experiment in human participants (n = 12). We used a saccadic mislocalization task as a behaviourally measureable proxy for the remapping process, and

simultaneously recorded 64-channel EEG. The saccadic mislocalization task was adapted to avoid abrupt transient events (such as a saccade cue) that could otherwise have affected ongoing oscillatory activity. We then used a time-frequency analysis (phase-opposition) to test for a correlation between the phase of oscillations at time points relative to saccade initiation and the perceptual outcome (amount of mislocalization) on a given trial. We are interested in the role of spontaneous ongoing oscillations, and so only time points prior to the saccade can be considered, since post-saccadic activity tends to be contaminated both by saccade-related reset of oscillations and by large-amplitude eye-movement related activity. We found significant phase-opposition in a time-frequency region of 400-300 ms prior to saccade initiation, and from 6 to 8 Hz (peak at -360 ms, 6.6 Hz), principally apparent over occipital electrodes. Put more simply, this demonstrates that the degree of perceived mislocalization is correlated with the phase of a theta-frequency oscillation prior to saccade onset. On average, there was 0.1 degrees more mislocalization following the 'best' phase than following the 'worst' (corresponding to about 10% of the average mislocalization amplitude). We conclude that saccadic remapping is indeed a rhythmic process. On the basis of the current data set, we cannot definitively determine whether this rhythmicity is intrinsic to the remapping process or inherited from underlying attentional processes. However, we note that the characteristics of the oscillatory process detected (time and frequency range) are strongly consistent with previous reports of rhythmic processes in attention.

Disclosures: D. McLelland: None. **L. Lavergne:** None. **R. VanRullen:** None.

Poster

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Topic: D.06. Eye Movements

Support: NIH Grant MH060358

Title: Laminar profiles of top-down vs bottom-up neuronal interactions during free viewing

Authors: *A. TRONGNETRPUNYA[1], A. BARCZAK[3], S. HAEGENS[4], C. E. SCHROEDER[5], M. DING[2]
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Abstract: Saccadic eye movements are essential for the active perceptual processing of visual scenes. Saccades partition visual input into chunks or “volleys” that course into the system at the onset of each fixation. Along with this “parsing” effect on retinal input, saccades also produce nonretinal modulation of visual cortical circuits. While a number of groups have reported these effects and several have hypothesized that nonretinal signals modulate the transfer of information across visual cortical stages, no one has tested this hypothesis directly. We examined the problem by analyzing laminar profiles of local field potentials (LFPs) recorded with concurrent linear array (laminar) multielectrodes from the visual cortex of two macaque monkeys performing a free viewing task where each image appeared on the screen for 5 seconds which was followed by a 3 second black screen break. For each eye movement, the saccade initiation period is defined as the 250ms period before saccade onset, and the fixation period is defined as the 250ms period after fixation onset. Applying Granger causality analysis we found that within V1, Granger causal influences from supragranular to granular layers in the alpha and theta range were higher in the saccade initiation period than the fixation period. Granger causal influences in the opposite direction, in the alpha and beta frequency range, were higher during the fixation period than the initiation period. Between cortical areas, Granger causal influences from V4 infragranular to V1 supragranular and infragranular layers were higher in the saccade initiation period than the fixation period in the theta, alpha and beta frequency range, while Granger causal influences from V1 granular to V4 infragranular layers were higher in the fixation period than the initiation period in the alpha and beta frequency range. These findings appear to support the notion that prior to saccade onset, top-down influences dominate in the visual hierarchy, whereas following fixation onset, cortical visual areas are primed to process bottom-up sensory input. Furthermore, the processes mediating feedforward and feedback interactions in both intra-area and inter-areal processing may use different oscillatory frequencies.

Disclosures: A. Trongnetrpunya: None. **A. Barczak:** None. **M. Ding:** None. **C.E. Schroeder:** None. **S. Haegens:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: Barrow Neurological Foundation

Title: Contrast-dependent responses to microsaccades in area V1

Authors: *J. CUI, S. L. MACKNIK, S. MARTINEZ-CONDE
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Abstract: Even during attempted fixation, our eyes are rarely still but produce a variety of eye movements. Such fixational eye movements include microsaccades, or small-magnitude saccades (typically < 1°) that occur once or twice a second during fixation on a target. Microsaccades evoke strong responses in area V1 neurons, but the nature of this neural activity is not well understood. Here we show variations in microsaccade-triggered single-unit activity in primate area V1 as a function of stimulus contrast. Three monkeys were trained to fixate a small red cross, while their eye movements were recorded using the scleral search coil technique. Visual stimuli consisted of circular patches of orientated gratings, presented at ten different levels of Michelson contrast. The stimuli were centered over the cells' receptive fields, with orientations matching the preferred orientation of each neuron. We found a prominent tri-phasic modulation of responses (i.e. an initial enhancement followed by decreased responses and a later enhancement), which varied in strength as a function of contrast.

Disclosures: J. Cui: None. **S.L. Macknik:** None. **S. Martinez-Conde:** None.

Poster

438. Eye Movements: Cortex

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Eye and Ear Foundation of Pittsburgh

Title: Structure of local field potential coherence within and between FEF and V4 during eye movement planning

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Abstract: Local field potentials (LFPs) reflect the synchronized synaptic currents of a small group of neurons. Specific frequency components of the LFP have been suggested to play an active role in cortical processing, and an increasing body of evidence has linked activity in the LFP to correlated fluctuations in neuronal spiking. Due to the resonant characteristics of recurrent neural networks, different frequencies in the LFP (and in turn spiking activity) are linked to different spatial scales of neuronal communication, such as within and between cortical areas. Teasing apart how populations of neurons communicate both within and across regions is particularly important to understanding perception and behavior. A means of analyzing these frequency components separately is through mean squared coherence, the frequency domain analog of correlation. Although coherence has been examined in early visual areas, little is known about the coherence of higher cortical areas such as the frontal eye fields (FEF) and visual cortex. We first investigated the magnitude squared coherence of the LFP separately within FEF and V4 as a function of frequency band and distance between recording sites. We predicted that the results in FEF and V4 would be consistent with those previously found in V1 and V2, namely that coherence across all frequency bands decreases as a function of distance. LFPs in FEF and V4 were recorded using a 16-channel linear probe and a 96-channel "Utah array", respectively, while an alert rhesus macaque monkey performed a conventional memory guided saccade task. We found coherence across all frequency bands decreased as a function of electrode contact distance for both FEF and V4. Using the same task, we next examined inter-region coherence between FEF and V4. We found coherence between FEF and V4 electrode sites was tuned for saccade direction, and this tuning was modulated by the LFP tuning preference of the FEF electrode site in a frequency-specific manner. We conclude that the structure of coherence within regions is conserved across much of neocortex and that between-region coherence is dependent on both the frequency range and tuning properties of the LFPs.

Disclosures: S.B. Khanna: None. **A. Snyder:** None. **M. Smith:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Support: NIH Grant EY019273

Title: Cognitive control of attentional priority by the frontal eye field

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Abstract: To find objects quickly and effortlessly in cluttered visual scenes, people recruit an efficient strategy to guide their saccades. To achieve this, we believe that the brain creates a priority map of the outside world in the lateral intraparietal area (LIP), which incorporates both pre-attentive and cognitive inputs to represent the importance of stimuli in the scene. Saccades are then made to the location on the map with the highest level of activity. We previously observed that the responses of LIP neurons to objects that had already been inspected were suppressed, suggesting a form of inhibitory tagging (Mirpour et al, 2009). Hasagawa et al (2004) showed that there is a subset of neurons in the Frontal Eye Field (FEF) that respond when a stimulus that should not be looked at is in their receptive fields. We hypothesized that these neurons would preferentially respond to previously fixated Ts and would do so during a time period that could explain the reduced responses in LIP. LIP activity is also thought to be correlated with the likelihood of reward or relative reward value during decision making, but we have previously found that LIP responses must be normalized before they correlate with behavior or reward across multiple conditions (Mirpour and Bisley, 2012). In this study we examined the responses of neurons in FEF to test whether a subset responds preferentially to previously fixated Ts and to see whether a subset had responses that matched the output of our proposed normalization process. Animals searched through 5 potential targets and 5 distractors to identify the target that was loaded with reward. After the stimuli appeared, the animals were free to move their eyes to find the reward-loaded target. Stimuli were spaced such that when the animal was looking at one stimulus, another was in the FEF neuron's receptive field. Most of the neurons fit into 3 main classes: neurons that responded preferentially to Ts; neurons that responded preferentially when a stimulus that had been fixated was in the response field and which could drive top-down inhibitory tagging; and neurons that initially showed an enhanced response to a stimulus that had been fixated, but that reversed their response preference around 100-150 ms after the end of the saccade. This latter class had responses that matched our post-normalization predictions after the response reversal. Together these data suggest that there is a functional reciprocal connection between LIP and FEF, which is involved in guiding saccadic eye movements during active, goal directed visual search.

Disclosures: K. Mirpour: None. **J.W. Bisley:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.06. Eye Movements

Title: Contributions of frontal eye field spiking activity and synchrony to control of eye movements

Authors: A.-H. VAHABIE[1], M.-R. A. DEHAQANI[1], C. SUN[2], B. NOUDOOST[4], *A. SOLTANI[3]
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Abstract: In order to collect information about the visual world, we move our eyes several times a second; the endpoints of these eye movements are precisely guided by the activity of a network of oculomotor cortical and subcortical areas. Frontal eye field (FEF) is a prefrontal cortex heavily implicated in the control of gaze. The goal of this study is to understand how neural activity in this area drives the accurate execution of saccadic eye movements. Specifically we wish to determine to what degree activity of various functional types of neurons and their interaction within this area contribute to guiding the gaze. We simultaneously recorded FEF activity from multiple neurons with a 16-channel linear electrode during a memory-guided saccade task, and use this activity to predict the saccade endpoints and accuracy. The monkeys were trained to make eye movements toward a previously presented visual target after a 1-second delay (delay period). The target could appear in one of N locations (N=2, 8, or 16) around the fixation point for 1 sec (cue period). We applied various classifications methods to spiking activity and synchrony between simultaneously recorded neurons to predict saccade endpoints. The results were threefold: First, as expected, we found that FEF population activity alone could predict saccade endpoints around the target location to a good extent. Second, we found that biases in eye position during fixation, cue, and delay periods were informative regarding the upcoming saccade endpoints. Interestingly, spiking activity during the fixation period was predictive of the bias in eye position before and during target presentation, and including this bias could dramatically improve endpoint estimation. Thirdly, the power spectrum of spiking activity and coherence between simultaneously recorded neurons reflected saccade accuracy. We found a decrease in alpha-band power and an increase in gamma-band power associated with more accurate saccades. Noise correlations between FEF neurons during the fixation period also

varied with subsequent saccade accuracy, with greater noise correlations preceding less accurate saccades. Our results identify potential neural substrates within FEF underlying the precise control of saccadic eye movements.

Disclosures: **A. Vahabie:** None. **M.A. Dehaqani:** None. **C. Sun:** None. **A. Soltani:** None. **B. Noudoost:** None.

Poster

438. Eye Movements: Cortex

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NIH Intramural Research Program

Title: V1 response variability driven by fixational eye movements

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Abstract: Understanding sensory neuron variability has broad implications for questions of cortical coding, and the relationship of neural activity to perception and behavior. In primary visual cortex (V1), the magnitude, structure, and state-dependence of neural variability remain strongly debated. Previous studies have shown that in awake primates, minimizing the contribution of small involuntary 'fixational' eye movements can have substantial impacts on measures of cortical variability. However, the precise contribution of fixational eye movements to such measures remains unknown, due in large part to an inability to reconstruct the retinal stimulus with sufficient accuracy using existing eye-tracking methods. Here we overcome this limitation using a recently developed method for inferring an animal's eye position with arc-minute accuracy using multi-electrode array recordings coupled with nonlinear stimulus processing models. We use planar and laminar multi-electrode arrays to record foveal and parafoveal V1 activity from awake, fixating macaques while presenting a one-dimensional 'ternary noise' stimulus. Combining our model-based eye tracking method and nonlinear stimulus processing models, we partition the stimulus-driven modulation of each neuron's firing

rate into a component due to changes in the displayed stimulus, and a component due to fluctuations in eye position. Surprisingly, eye movements contributed well over half the response variance in most neurons, such that the variance of 'PSTHs' measured in response to repeated presentations of the same stimulus were under-estimated by more than a factor of two on average. This eye-movement induced response variability was larger for neurons that had smaller receptive fields (RFs) and higher preferred spatial frequencies (as in the fovea), but remained substantial even for neurons recorded outside the fovea. We also show that, in the presence of fixational eye movements, standard procedures for estimating 'noise correlations' lead to large biases, resulting in strong artifactual relationships between noise correlations and RF similarity. This work thus shows that precise tracking of fixational eye movements is critical for studies of V1 neuron variability in awake animals.

Disclosures: J.M. McFarland: None. **A.G. Bondy:** None. **B.G. Cumming:** None. **D.A. Butts:** None.

Poster

438. Eye Movements: Cortex

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Support: NSF Graduate Research Fellowship

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Title: Circuits underlying covert attention and saccade preparation within the primate frontal eye field

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Abstract: Primates investigate environments visually by shifting gaze and focus of attention several times every second. Decisions about where to shift gaze and attention incorporate both exogenous (visual) and endogenous (goal-related) types of information. The frontal eye field (FEF) is a region of frontal cortex crucial for performing these behaviors. Neurons within the

FEF represent visual, eye movement-related (i.e. saccadic), and attention-related task variables. A central question for understanding the neural circuit mechanisms underlying decision making is whether neurons with different response properties play distinct functional roles in serial processing chains or whether they instead represent aspects of underlying dynamical computations or exhibit mixed selectivity as an advantageous computational mechanism. We investigated the circuits underlying gaze and attention shifts by training monkeys (Macaca mulatta) to dissociate the location of covert spatial attention from the target of saccades. In this task, monkeys were cued to attend one of four peripheral stimuli without shifting their gaze to it. After a delay, one of the four stimuli changed orientation, and monkeys were rewarded upon making a saccade to the opposite stimulus from the change location (antisaccade response). On other trials, no stimuli changed and monkeys were rewarded for continued fixation. Monkeys thus attended one stimulus while planning a saccade to another. We recorded the activity of 421 neurons in the FEFs of two monkeys using linear array electrodes (median 20 neurons per recording). We found that, unexpectedly, while many neurons represented the location of covert attention or of saccade plans, these representations were independent of their visual and saccade-related representations. By behaviorally separating two related functions of FEF circuits, we have revealed that FEF neurons may not serve consistent roles in a simple circuit but rather support behavior via mixed coding.

Disclosures: **N.A. Steinmetz:** None. **T. Moore:** None.

Poster

438. Eye Movements: Cortex

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Title: Visually induced gamma-band activity is entrained to rhythmic modulation of behavioral relevance

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Abstract: In everyday natural vision, our visual system is constantly presented with the task of selecting and attending to behaviorally relevant stimuli at the expense of others. Endogenous rhythms, such as the gamma-band (30-100Hz), have been proposed to mediate the flow of incoming sensory information by rhythmically modulating the local input gain (Fries, 2005, Trends in Cognitive Sciences). These endogenous rhythms may in turn be influenced by external rhythms present in the incoming sensory information - especially if those external rhythms provide behaviorally relevant temporal information, such as the moment of an anticipated stimulus change. We recorded local field potentials (LFPs) with electrocorticography (ECoG) grids implanted subdurally with a 252 sites covering V1 and V4 among other areas in two macaques while they selectively attended to one of two simultaneously presented moving grating stimuli, and responded to a change in the attended grating while ignoring a change in the unattended (Bosman et al., 2012, Neuron). The behaviorally relevant change always occurred at the same phase of the grating cycle. Thus, the stimuli contained behaviorally relevant rhythmic information, and the animals learned to use this information to anticipate moments when the attended stimulus was most likely to change. We observed that this external rhythm resulted in a rhythmic modulation of the microsaccade rate, such that microsaccades were less likely to occur during moments when the change in the attended stimulus was most likely to occur. The gamma-band (~60-80Hz) activity in V1 and V4 was also modulated by this stimulus-change-anticipatory rhythm. Intriguingly, the modulation of endogenous rhythms by the stimulus-change-anticipatory rhythm was not independent of attention. That is, the rhythm of the attended stimulus, not the unattended stimulus, appeared to modulate the gamma in V1 and V4 for both the attended and unattended stimuli. These results suggest that endogenously generated gamma-band synchronization and its entrainment to exogenous stimulus rhythms can operate synergistically to implement attentional selection of rhythmic stimuli.

Disclosures: J.R. Dowdall: None. **C.A. Bosman:** None. **P. Fries:** None.

Poster

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ORF (Canada)

Title: HD-tDCS modulates decision response times

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Abstract: Decision making processes in the brain are often modeled by bounded accumulator models, which specify that noisy evidence is integrated over time until reaching a decision boundary in favor of one alternative. Theoretical work by Wong and Wang (2006) predicts that a common excitatory input to the integrator populations can alter the network dynamics. Briefly, the stronger the excitation, the faster the network can accumulate sensory evidence to reach a decision. Thus, faster dynamics should result in decreased decision latencies while slower dynamics should result in increased latencies. Conveniently, high-density transcranial direct current stimulation (HD-tDCS) has been theorized to influence the excitability of neural circuits by altering the resting potential of neuronal membranes. Generally, when current flows in the anodal (cathodal) direction, neurons become more (less) sensitive to excitatory input. Here, we investigated the effect of HD-tDCS on decision response times using a 2-AFC random dot motion direction discrimination reaction time task during stimulation (anodal current, cathodal current or no stimulation) over the human right caudal middle frontal area (encompassing the frontal eye fields), which receives inputs from temporal and parietal motion processing areas active during visuomotor tasks and is thought to be heavily involved in the planning of eye movement decisions. Human participants determined the direction (left or right) of a randomly selected subset of coherently (2, 10 or 20% coherence) moving dots and responded with a left/right saccade. Across all coherence levels, latencies were consistently shorter under anodal currents compared to latencies under cathodal currents for both leftward and rightward movements, suggesting faster decision dynamics with anodal (excitatory) tDCS. Compared to no stimulation, at 10% coherence anodal currents decreased average (± SD) response latencies by 183 ms (± 208 ms) and cathodal currents increased response latencies by 37 ms (± 238 ms). However, at 20% coherence, both current directions decreased response latencies (170 ms (± 81

ms) for anodal and 61 ms (± 119 ms) for cathodal). Also, lateralized latency effects varied with coherence. Rightward latencies were 352 ms (± 451 ms) longer than leftward latencies at 2% coherence, but 57 ms (± 250 ms) and 87 ms (± 95 ms) shorter at 10 and 20% coherence respectively. This suggests a facilitation of decisions in the direction contralateral to the stimulation location under easy conditions. Thus, the dynamics of the decision circuitry can be modulated by HD-tDCS in a manner consistent with predictions of established models.

Disclosures: **T. Murdison:** None. **O.Y. Lee:** None. **D. Standage:** None. **G. Blohm:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.05. Visual Sensory-motor Processing

Support: NSF BCS1261433

NIH T32 EY018080

Title: Natural vision effects on V1 activity and contrast sensitivity

Authors: ***J. NIEMEYER**, M. PARADISO
Brown Univ., Providence, RI

Abstract: The measurement of contrast sensitivity (CS) is a powerful tool both for characterizing visual processing and for quantifying visual deficits. For this reason it is important to understand what factors influence CS and obtain measurements that reflect natural vision as accurately as possible. Motivated by previous physiological data from our lab, we investigated the hypothesis that the exploratory saccades and complex scenes typical of natural vision are associated with contrast sensitivity that differs from that obtained in more simplified situations. Many vision studies are rather unnatural in that the stimuli are simple rather than complex and the stimuli are brought into view by flashing them after a period of fixation instead of them being acquired by saccades. Our previous research showed that stimuli brought into V1 receptive fields (RFs) by a saccade produce a significantly lower response than the same stimulus flashed into view. In the present study we have recorded V1 responses to Gabor stimuli with a range of contrasts on a complex natural image background at the same time that animals performed a 2AFC contrast detection task. The stimuli appeared either by being flashed in the RF or as a

result of a saccade. We find that V1 responses are significantly lower to stimuli that come into the RF by a saccade compared to stimuli that are flashed. This reduction in neural sensitivity is strong at lower spatial frequencies and decreases at higher frequencies. In the behavioral data, the contrast sensitivity functions (CSFs) show that, after saccades, perceptual sensitivity is reduced and this is mainly true at lower spatial frequencies. There is good correspondence between the stimulus parameters under which the neural and perceptual effects are found, and interestingly, the effective parameters are not consistent with those that yield saccadic suppression. In additional experiments we altered the background stimulus to explore the role that the natural background image plays in the CSF and V1 response changes. The results suggest that part of the explanation for the reductions in the CSF and V1 activity is rapid neuronal adaptation to the natural scene on the fixation prior to the test fixation. This spatial-frequency-specific adaptation appears to play a role in determining contrast sensitivity after each saccade. In summary, the results demonstrate that several aspects of natural vision alter V1 activity and the CSF; this finding may have significant implications for clinical vision assessment. In related psychophysical studies, our lab has found that human CS measured in a more natural paradigm is consistent with the findings in the nonhuman primates.

Disclosures: J. Niemeyer: None. **M. Paradiso:** None.

Poster

438. Eye Movements: Cortex

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Topic: D.05. Visual Sensory-motor Processing

Support: Trent University

Title: Repetitive transcranial magnetic stimulation to the dorsal premotor cortex and near-hand effects

Authors: *S. CARLIN, L. E. BROWN
Trent Univ., Peterborough, ON, Canada

Abstract: Near-hand benefits are seen when individuals are able to process targets more quickly, accurately, and with greater precision when a hand is placed near, rather than far from a target. Differing views exist about the source of near-hand benefits. One possibility is that items appearing near the hand are prioritized for attentional selection because the hand acts as a cue for

the visual-spatial attention. Another explanation is that items appearing near the hands recruit visual-tactile bimodal cells. These cells are activated in response to either visual or tactile stimuli presented on or near the skin. The purpose of this experiment is to reveal the psychophysical and neural basis of hand-proximity effects in humans. Previous research demonstrating hand-proximity effects on eye-movement initiation reports that placing a hand near a target delayed the onset of immediate (reflexive) saccades and speeded the onset of delayed (volitional) saccades. In the current study, we extend previous findings by examining the timing of saccade initiation to targets appearing near a real hand, a realistic fake hand, or a non-hand visual cue. To establish the link between near-hand benefits and bimodal cells, rTMS was used to depress cortical activity in the left dorsal premotor area (PMd), a region thought to house bimodal cells. It was predicted that if these differences in saccade initiation are due to the recruitment of bimodal cells, near-hand effects would be observed in the real-hand condition only. Our initial analysis supports this prediction to an extent: real hand-proximity slowed both immediate and delayed saccades. The discussion will focus on the relative contributions of rTMS, bimodal cells and attention to near-hand effects.

Disclosures: S. Carlin: None. **L.E. Brown:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 439.01/II4

Topic: D.08. Pain

Support: NSC grant NSC102-2321-B-008-001

Title: Changes of DRG neuron population in neuropathic pain

Authors: T.-Y. LEE, C.-C. KUNG, *W.-H. SUN
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Abstract: Neuropathic pain, defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system, is one of the most devastating forms of chronic pain. The development of neuropathic pain involves activation of astrocytes and microglia in the spinal cord. Some studies have shown that peripheral nerve injury induces an early spinal microglial activation that precedes astrocytic activation. Previous studies found that chronic constriction injury (CCI) of sciatic nerve in mice caused chronic neuropathic pain for more than 14 weeks

and the gene expression of G2A and TDAG8, two of the proton-sensing G-protein-coupled receptors (GPCRs), was up-regulated in the CCI mice. In our study, sections of the lumbar 5 (L5) dorsal root ganglia (DRG) were processed for immunohistochemistry using antibodies against peripherin (PERI), and neurofilament 200 (N52) to identify small- and large-diameter primary sensory afferent neurons respectively, as well as using *in situ* hybridization to identify the localization of G2A and TDAG8. Previous study found that the expression of G2A and TDAG8 were up-regulated after CCI. Our data indicated that the relative proportion of small-diameter neurons labeled by PERI was slightly decreased 1 week and considerably decreased 14 weeks after the surgery, while the relative proportion of large-diameter neurons was increased 14 weeks after CCI. Therefore, it is likely that up-regulated expression of these proton-sensing GPCRs is due to increase number of large-diameter neurons, microglia, or astrocytes.

Disclosures: **T. Lee:** None. **C. Kung:** None. **W. Sun:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

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Topic: D.08. Pain

Support: NIH Grant DE018573

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Title: 5-HT-dependent descending facilitation maintains long-lasting hyperactivity of dorsal horn astrocytes but not microglia and contributes to the maintenance and spread of neuropathic pain

Authors: J.-L. YANG, W. GUO, *Y.-X. CHU, S. ZOU, K. REN, R. DUBNER, F. WEI
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Abstract: Evidence has demonstrated that glial hyperactivity/reactivation and subsequent glia-neuronal interactions in the medullary and spinal dorsal horns after nerve injury are involved in central sensitization underlying the development of persistent pain. Our recent studies showed that 5-HT-dependent descending facilitation from the rostral ventromedial medulla (RVM) contributes to the maintenance of neuronal hyperexcitability in the dorsal horn and the

persistence of secondary hyperalgesia. In the present study, we investigated the effect of 5-HT-dependent descending facilitation on glial cell activity in the trigeminal subnucleus caudalis (Vc) during the maintenance of neuropathic pain after CCI of the infraorbital nerve (ION) in the rat. Western blot analysis showed long-lasting upregulation of astrocytic marker protein GFAP and microglial CD11b in the ipsilateral Vc after CCI. Immunostaining demonstrated a similar pattern of enhanced GFAP and CD11b expression for both microglia and astrocytes in the superficial and deep layers of the ipsilateral Vc V2 and V3 subregions at 5d and 14d after CCI. However, molecular depletion of 5-HT from RVM neurons by local gene transfer of Tph-2 shRNA significantly suppressed upregulation of GFAP but not CD11b and Iba-1 expression in the Vc at 14 d after nerve injury, suggesting that the maintenance of prolonged upregulation of GFAP in the VC after trigeminal nerve injury may depend on a predominant switch of excitatory input from the injured peripheral nerve to 5-HT-dependent descending facilitation. We further examined temporal patterns of GFAP and Iba-1 expression after CCI by co-labeling with transcription factors c-Fos or FosB, molecular markers for transient (hours) or long-term (days to weeks) adaptation of neuronal and glial activity, respectively. We did not observe c-Fos protein expression in GFAP or Iba-1 expressed glial cells in the Vc at 5 d and 14 d time points after CCI. In contrast, the number of FosB-expressing astrocytes in the Vc was significantly increased at the 14 d time point when compared to that at 5 d after CCI-ION. Depletion of descending 5-HT from the RVM decreased Fos-B expression in the Vc at 14 d. In addition, intra-Vc microinjection of the selective astrocytic inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, 100 nmol) significantly blocked CCI-induced secondary hyperalgesia at 14 d after CCI-ION. These data indicate that long-lasting hyperactivation of astrocytes in the dorsal horn predominately contributes to glial mechanisms of 5-HT-dependent descending facilitation underlying the maintenance of neuropathic pain that spreads to areas outside the zone of injury.

Disclosures: **J. Yang:** None. **Y. Chu:** None. **W. Guo:** None. **S. Zou:** None. **K. Ren:** None. **R. Dubner:** None. **F. Wei:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

Location: Halls A-C

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Program#/Poster#: 439.03/II6

Topic: D.08. Pain

Title: Astrocytes-specific transcriptional activation of MCP-3 associated with histone modifications in the spinal cord by peripheral neuropathy

Authors: *D. IKEGAMI[1], K. OHI[1], M. NARITA[1], N. KUZUMAKI[1], T. USHIJIMA[2], M. NARITA[1,3]
[1]Dept. Pharmacol, Hoshi Univ., Tokyo, Japan; [2]Div. of Epigenomics, Natl. Cancer Ctr. Res. Institute., Tokyo, Japan; [3]Life Sci. Tokyo Advanced research center (L-StaR), Tokyo, Japan

Abstract: The mechanism of neuropathic pain accompanied by long-lasting changes in pain transmission remains unclear. In the present study, we found that partial sciatic nerve ligation most remarkably increased the expression of monocyte chemotactic protein 3 (MCP-3, known as CCL7) in the spinal cord, which lasted for 4 weeks. This increase was accompanied by the decreased trimethylation of histone H3 at lysine27 (H3K27me3) at the promoter for 4 weeks after nerve ligation. In agreement with these data, sciatic nerve ligation significantly increased the induction of a Jumonji domein containing 3 (Jmjd3) at MCP-3 promoter. The robust increases in MCP3 in the dorsal horn of the spinal cord with partial sciatic nerve ligation were seen mostly in astrocytes. Consistent with immunohistochemistry assay, our magnetic-activated cell sorting assay revealed that the expression of MCP-3 mRNA was significantly increased in GLAST-positive astrocyte cells, but not in oligodendrocyte/neuron population or GLAST-negative microglia population obtained from the spinal cord of mice with nerve ligation. Interestingly, microinjection of sh-jmjd3 into the spinal cord was effective for reversing the neuropathic pain-like behavior following nerve injury. These findings suggest that increased MCP-3 expression associated with epigenetic modification at the MCP-3 promoter after nerve injury, mostly in spinal astrocytes, may serve to facilitate the spinal-sensitization and could play a critical role in the neuropathic pain-like state. Furthermore, the blockade of jmjd3 within the spinal cord may be an effective approach to relieve the neuropathic pain.

Disclosures: D. Ikegami: None. **K. Ohi:** None. **M. Narita:** None. **N. Kuzumaki:** None. **T. Ushijima:** None. **M. Narita:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

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Title: Therapeutic morphine prolongs neuropathic pain in rats: A role for TLR4 and inflammasome signaling in the lumbar spinal cord

Authors: *P. M. GRACE[1], K. A. STRAND[1], E. L. GALER[1], Y. ZHANG[1], D. BERKELHAMMER[1], L. I. GREENE[1], K. C. RICE[2], S. F. MAIER[1], L. R. WATKINS[1]
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Abstract: Recent clinical studies, as well as rodent studies in our lab, revealed a previously unsuspected deleterious effect of treating pain with opioids. Despite their status as gold-standard therapeutic analgesics for neuropathic pain, opioids initiate microgliosis and pronociceptive cytokine release via Toll Like Receptor 4 (TLR4) in naïve animals. However, the behavioral and molecular impact of opioid-induced gliosis has not been defined in the presence of peripheral nerve injury, which also induces lumbar spinal gliosis per se. To address this clinically relevant question, we hypothesized that sciatic chronic constriction injury (CCI)-allodynia would be enhanced by subsequent repeated morphine in male F344 rats, involving TLR4, P2X7 receptor (P2X7R) and caspase-1, facilitating release of interleukin (IL)-1β. Beginning 10 days after CCI, morphine (5 mg/kg b.i.d.) or equivolume saline was administered for 5 days. Compared to vehicle, morphine significantly prolonged the duration of CCI-induced allodynia (n=6/group; $p<0.05$). Morphine also significantly elevated P2X7R, NFkappaB, NLRP3 and caspase-1 protein levels ($p<0.05$), and TLR4 and IL-1beta mRNA ($p<0.05$), in the ipsilateral lumbar dorsal quadrant (iLDQ), 5 weeks after dosing conclusion. Supporting a causal role for NLRP3 inflammasome activation in morphine-prolonged CCI-allodynia, continuous intrathecal infusion of inhibitors of TLR4 ([+]-naloxone; 60 μg/h), P2X7R (Brilliant Blue G; 30 ng/h), or caspase-1 (ac-YVAD-cmk; 1 μg/h) prevented prolonged allodynia when administered concomitantly with morphine, and abolished established morphine-prolonged CCI-allodynia when administered 5 weeks after morphine dosing (n=6/group; $p<0.05$). A single intrathecal IL-1 receptor antagonist dose (100 μg) also attenuated morphine-prolonged CCI-allodynia (n=6/group; $p<0.05$). In keeping with known pro-nociceptive roles for IL-1β, phosphorylation of the NR1 NMDA subunit was elevated, while GRK2 and GLT-1 levels were decreased in iLDQ 5 weeks after dosing conclusion ($p<0.05$). [+]-Naloxone coadministration during morphine treatment was sufficient to normalize P2X7R, NFkappaB, NLRP3, caspase-1, NR1, GRK2 and GLT-1 protein levels ($p<0.05$), and TLR4 and IL-1beta mRNA ($p<0.05$) in the iLDQ, 5 weeks after dosing. These data suggest that morphine and the products of nerve injury interact, resulting in prolonged neuropathic pain via sustained inflammasome signaling. These data present an opportunity to pharmacologically inhibit paradoxical pain enhancement while retaining the analgesic properties of morphine, as each is mediated via different receptors and underlying mechanisms.

Disclosures: **P.M. Grace:** None. **K.A. Strand:** None. **E.L. Galer:** None. **Y. Zhang:** None. **D. Berkelhammer:** None. **L.I. Greene:** None. **K.C. Rice:** None. **S.F. Maier:** None. **L.R. Watkins:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 439.05/II8

Topic: D.08. Pain

Support: KAKENHI Grant No.23229008

Title: Spinal astrocytic STAT3 is a crucial factor for reactive astrocytes in neuropathic pain

Authors: ***Y. KOHRO**[1], M. TSUDA[1], E. SAKAGUCHI[1], H. TOZAKI-SAITOH[1], H. OKANO[2], K. INOUE[1]
[1]Dept. Mol. Syst. Pharmacol., Grad Sch. Pharmaceut Sci., Kyushu Univ., Fukuoka, Japan; [2]Dept. Physio., Keio Univ. Sch. Med., Tokyo, Japan

Abstract: Neuropathic pain is a debilitating pain state, which is often caused by lesion or dysfunction in the nervous system arising from cancer, diabetes, herpes zoster or surgery. Accumulating evidence indicates that spinal astrocytes become reactive states in chronic pain states from rodents to humans, which is a crucial role for neuropathic pain. However, how to converting from resting astrocytes to reactive astrocytes after peripheral nerve injury (PNI) are largely unknown. We have previously shown that STAT3, a transcription factor which is activated predominantly in spinal astrocytes after PNI plays an important role in the maintenance of neuropathic pain. The aim of present study is to investigate the precise role of astrocytic STAT3 signalling in chronic pain using mice with conditional deletion of STAT3 in astrocytes (GFAP-Cre/STAT3fl/fl: STAT3-cKO). We found that disruption of astrocytic STAT3 prevented activation of STAT3 and upregulation of expression of genes related with reactive state of astrocytes following PNI. Moreover, STAT3-cKO mice showed the resistance and shortened duration of PNI-induced tactile allodynia, a typical symptom of neuropathic pain. To further confirm of the role of spinal astrocytic STAT3 signalling, we injected adeno-associated viruses to control STAT3 activity in spinal dorsal horn astrocytes. We found that introducing dominant negative form of STAT3 in spinal astrocytes suppressed upregulation of genes related with reactive astrocytes and tactile allodynia after PNI. In addition, forced expression of constitutive active STAT3 in spinal astrocytes induced tactile allodynia. Together, our findings suggest that

STAT3 is a key regulator of converting reactive states of spinal astrocytes that drive neuropathic pain.

Disclosures: **Y. Kohro:** None. **M. Tsuda:** None. **E. Sakaguchi:** None. **H. Tozaki-Saitoh:** None. **H. Okano:** None. **K. Inoue:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

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Support: Université de Strasbourg

CNRS

INSERM

GLORIA, FP7 Grant n°: 602919

Title: Activation of astrocytes by sciatic nerve ligation-induced neuropathic pain in delta opioid receptor knockout and wild type mice

Authors: *C. GAVERIAUX-RUFF[1,2], H. MAURIN[3], D. REISS[3], L.-A. ROECKEL[3], B. L. KIEFFER[3]
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Abstract: Aim of investigation: Neuropathic pain triggered by partial sciatic nerve ligation (pSNL) has been shown to activate astrocytes and microglia in spinal cord and astrocytes in brain regions linked to emotional responses like cortex and amygdala. A role for delta opioid receptor has been suggested, based on the treatment by the delta opioid antagonist naltrindole. We have investigated the role of delta opioid receptor endogenous tone in the activation of astrocytes and microglia induced by pSNL neuropathy. Methods: To investigate delta opioid receptor implication in the activation of glial cells induced by partial sciatic nerve ligation, we have compared the expression of the astrocyte activation marker Gliary Fibrillary Acidic Protein (GFAP) as well as of the microglia activation markers Iba-1 and CD11b, in both L4-L6 spinal

cord and brain following neuropathy in delta receptor knockout and wild-type mice. Results: Delta opioid receptor knockout (KO) mice display normal nociceptive thresholds, indicating that endogenous delta opioid tone is not a major controller of acute pain perception. However, delta receptor KO animals displayed augmented mechanical allodynia under neuropathic pain condition, evidencing an endogenous delta opioid tone for neuropathy-induced hypersensitivity. Astrocytes and microglia are shown to be activated by neuropathy. The comparison of delta receptor mutants and wild-type animals will indicate delta receptor implication in glial cell activation. Conclusions : The data obtained from delta receptor KO mice highlight the important implication of delta receptor in chronic neuropathic pain development and maintenance. Together with glia activation results, it strengthens the idea of a role for delta receptors expressed by glial cells in novel strategies against chronic pain and associated emotional diseases.

Disclosures: C. Gaveriaux-Ruff: None. **H. Maurin:** None. **D. Reiss:** None. **L. Roeckel:** None. **B.L. Kieffer:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 439.07/II10

Topic: D.08. Pain

Title: Changes of microglia activity by basic fibroblast growth factor on neuropathic pain

Authors: H. FUJIMAKI[1], *G. INOUE[2], K. UCHIDA[1], W. SAITO[1], H. SEKIGUCHI[1], N. TAKAHIRA[1], M. TAKASO[1], G. INOUE[1]
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Abstract: Basic fibroblast growth factor (bFGF) is a growth factor which is known to regulate angiogenesis and arteriogenesis. The bFGF also promotes proliferation and differentiation on neural progenitor cell and is also a trophic factor for neurons and astrocytes. Recently, the influence of bFGF as a transmitter of pain is reported. In this study, we evaluated the expression of bFGF and glial activation in rats spinal nerve ligation model and investigated the potential of anti-bFGF neutralizing antibodies as a treatment of the neuropathic pain. Sprague-Dawley rats (6 weeks in age) which L5 spinal nerve was ligated were used. To evaluate the contribution of bFGF for neuropathic pain, we injected neutralizing antibodies of bFGF (40ul) intrathecally twice a week (experimental group). Rats were injected PBS as control (control group). Behavioral testing was evaluated by von Frey filament at 1,3,5,7,14,21 and 28 days after the

surgery. Ligated left L5 dorsal root ganglion (DRG) and spinal cord were processed to analyze for immunohistochemistry, western blotting and RT-qPCR assays for bFGF, and Iba1, which is marker of microglia or macrophage. In behavioral testing, allodynia was suppressed from postoperative 2 days to 4 weeks in experimental group. Also, bFGF and Iba1 expression in spinal cord was suppressed in both mRNA and protein. In immunohistochemistry, Iba1 expression in dorsal horn was significantly suppressed. The numbers of microglia were significantly smaller in experimental group in dorsal horn. bFGF is reported as an important growth factors for healing responses after tissue damage, such as nerve degeneration. On the other hand, in neuropathic pain state, bFGF was released from presynapticly, and activates glial cells, contributing the central sensitization. Based on our results, neutralizing antibody was suppressed allodynia and glial activation, suggesting bFGF may play an important role in the chronic neuropathic pain, and anti-bFGF treatment may facilitate recovery of neurological function and involve in microglial activity. bFGF could be one of the therapeutic target for the treatment of chronic neuropathic pain.

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Poster

439. Mechanisms of Neuropathic Pain: Glia

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Topic: D.08. Pain

Support: Medtronic Research Grant

Title: Microglial cell activation in the spinal dorsal horn: The pain-relieving effect of spinal cord stimulation in an experimental model of mono-neuropathy

Authors: M. VAN BEEK, *B. JOOSTEN, E. A. JOOSTEN, J. HEUSCHEN
Anesthesiol., Maastricht Univ. Med. Ctr., Maastricht, Netherlands

Abstract: Aims: In this study we investigated the effect of Spinal Cord Stimulation (SCS) on microglial cell activity in a rat model of mononeuropathy. Microglial cell activity is suggested to be related to the onset and maintenance of neuropathic pain. We hypothesize that SCS will de-acitivate the microglial cells and this is a part of the mechanism underlying the pain relieving effect of this treatment modality. Methods: Partial ligation of the sciatic nerve in adult Sprague

Dawley rats resulted in tactile hypersensitivity as assessed with von Frey filaments. A small monopolar electrode was epidurally implanted by a small laminectomy at T13 two weeks post injury. Subsequently, the animals were allowed to recover for two days after the electrode implantation. The stimulation paradigm consisted of assessment of motor threshold followed by 30 minutes of SCS (2/3 of motor threshold, frequency 2 Hz, pulse width 0.2 ms). Animals were sacrificed 30 or 90 minutes after cessation of SCS and spinal cord tissue was collected for immunohistochemical analyses of microglial cell markers Iba-1 (microglial morphology) and P-p38 MAPK (microglial cellular activation) in the dorsal horn. The behavioral pain relieving effect of SCS was analyzed by its effect on mechanical hypersensitivity with withdrawal response and von Frey filaments. Results: Partial ligation of the sciatic nerve resulted in a significant increase in Iba-1 gray value and area fraction in the dorsal horn. No differences in Iba-1 staining were observed in rats receiving SCS compared to sham operated animals, regardless of behavioral response to treatment (70% responder n=11, 30% non-responder n=5). The quantitative immunocytochemical analysis of cellular activation of microglial cells in the dorsal horn is presently executed. Conclusions: Partial ligation of the sciatic nerve results in microglial cell morphological changes (swelling) in the dorsal horn. SCS treatment did not affect Iba-1 staining intensity in the spinal dorsal horn. The cellular (de-)activation of the microglial cells due to SCS treatment is presently investigated.

Disclosures: **M. van Beek:** None. **B. Joosten:** None. **E.A. Joosten:** None. **J. Heuschen:** None.

Poster

439. Mechanisms of Neuropathic Pain: Glia

Location: Halls A-C

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Program#/Poster#: 439.09/II12

Topic: D.08. Pain

Support: CREST

NEXT Program

Title: IRF5 is a crucial determinant for the formation of P2X4R+ reactive microglia driving neuropathic pain

Authors: ***T. MASUDA**[1], S. IWAMOTO[1], R. YOSHINAGA[1], H. TOZAKI-SAITOH[1], A. NISHIYAMA[2], T. W. MAK[3], T. TAMURA[2], M. TSUDA[1], K. INOUE[1]

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Abstract: Microglia are the principal immune cells of the central nervous system (CNS). In response to neuronal injury or disease, microglia adopt distinct reactive phenotypes via the expression of different sets of genes and play crucial roles in diverse CNS pathologies. Spinal microglia that express the purinergic P2X4 receptor (P2X4R+ microglia) after peripheral nerve injury (PNI) are implicated in neuropathic pain. Here we show that interferon regulatory factor-5 (IRF5), an IRF family of transcription factor upregulated in spinal microglia after PNI, is responsible for direct transcriptional control of P2X4R. Upon stimulation of microglia by fibronectin, IRF5 induced de novo expression of P2X4R by directly binding to the promoter region of the P2rx4 gene. The deficiency of IRF5 abrogated PNI-induced upregulation of spinal P2X4R. Accordingly, IRF5-deficient mice showed substantial resistance to PNI-induced pain hypersensitivity. Furthermore, we found that expression of IRF5 in microglia is directly regulated by IRF8. Together, our findings indicate that the IRF8-IRF5 transcriptional axis may contribute to shifting microglia toward a P2X4R-expressing reactive state. These results may provide a new target for treating neuropathic pain.

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Poster

439. Mechanisms of Neuropathic Pain: Glia

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Topic: D.08. Pain

Support: NIH Grant R01DE22743-01

Title: Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice

Authors: *G. CHEN[1], C.-K. PARK[1], R.-G. XIE[1], T. BERTA[1], M. NEDERGAARD[2], R.-R. JI[1]
[1]Sensory Plasticity Laboratory/Department of Anesthesiol., Duke Univ. Med. Ctr., Durham, NC; [2]Ctr. for Translational Neuromedicine, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Accumulating evidence suggests that spinal cord astrocytes play an important role in neuropathic pain sensitization by releasing astrocytic mediators (e.g. cytokines, chemokines, and growth factors). However, it remains unclear how astrocytes control the release of astrocytic mediators and sustain neuropathic pain in the late phase. The connexin-43 (Cx43) is typically expressed by astrocytes and has been implicated in gap junction communication and the development of neuropathic pain. In this study, we investigated whether nerve injury could upregulate Cx43 to sustain late-phase neuropathic pain via releasing chemokine from spinal astrocytes. Chronic constriction injury (CCI) elicited a persistent up-regulation of Cx43 in spinal astrocytes for > 3 weeks. Spinal (intrathecal) injection of carbenoxolone (CBX, a non-selective hemichannel blocker) and selective Cx43 blockers (Cx43 mimetic peptides ^{43}Gap26 and ^{37,43}Gap27), as well as astroglial toxin but not microglial inhibitors, given 3 weeks after nerve injury, effectively reduced mechanical allodynia, a cardinal feature of neuropathic pain in the late-phase. In cultured astrocytes, TNF-α elicited marked release of the chemokine CXCL1, and the release was blocked by CBX, Gap26/Gap27, and Cx43 siRNA. TNF-α also increased Cx43 expression and hemichannel activity but not gap junction communication in astrocytes. Spinal injection of TNF-α-activated astrocytes is sufficient to induce persistent mechanical allodynia, and this allodynia was suppressed by CXCL1 neutralization, CXCL1 receptor (CXCR2) antagonist, and pre-treatment of astrocytes with Cx43-siRNA. Furthermore, nerve injury persistently increased excitatory synaptic transmission (EPSCs) in spinal lamina IIo nociceptive synapses in the late-phase, and this increase was suppressed by CBX and Gap27 and recapitulated by CXCL1. Together, our findings demonstrate a novel mechanism of astrocytic Cx43 to enhance spinal cord synaptic transmission and maintain neuropathic pain in the late-phase via releasing chemokines.

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Poster

439. Mechanisms of Neuropathic Pain: Glia

Location: Halls A-C

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Program#/Poster#: 439.11/II14

Topic: D.08. Pain

Support: NIH Grant NS064289

Title: Paclitaxel induces acute pain in rodents via activating microglial TLR4 and releasing IL-1 beta in the dorsal horn

Authors: *H.-R. WENG[1], X. YAN[2], D. MAIXNER[2], R. YADAV[2]
[1]Dept Pharmaceut. and Biomed. Sci., Univ. of Georgia Col. of Pharm., Athens, GA; [2]Dept. of Pharmaceut. and Biomed. Sci., The Univ. of Georgia Col. of Pharm., Athens, GA

Abstract: Paclitaxel (taxol) is a first-line chemotherapeutic-agent used for treatment of many types of cancers. Patients receiving taxol treatment often develop pathological pain, which significantly reduces the quality of life in patients and hampers the use of this otherwise life-saving chemotherapy in the clinic. Pathological pain induced by taxol in patients includes pain that occurs immediately after taxol treatment (known as paclitaxel-associated acute pain syndrome, P-APS), and pain that persists for weeks to years after cessation of paclitaxel treatment (known as paclitaxel induced chronic neuropathic pain). There is no proven standard of care for the prevention or treatment of P-APS and the mechanisms by which paclitaxel induces P-APA are not known. In this study, we found that paclitaxel causes acute pain in rodents in a dose-dependent manner. The paclitaxel-induced acute pain occurs within 2 hrs after a single intravenous injection of paclitaxel. This is accompanied with low levels of paclitaxel penetrating into the cerebral spinal fluid and spinal dorsal horn. We provided evidence that paclitaxel directly acts on microglial toll like receptor 4 (TLR4) and increases Ca2+ levels in microglia in the spinal dorsal horn, which in turn activates astrocytes through releasing interleukin-1β (IL-1β) IL-1β increases glutamatergic synaptic activities and reduces glial glutamate transporter activities in the spinal dorsal horn. Activations of TLR4 and IL-1β receptors are necessary in the genesis of paclitaxel-induced acute pain. The cellular and molecular signaling pathways revealed in this study could provide rationales for the development of analgesics and management strategies for P-APS in patients.

Disclosures: H. Weng: None. **X. Yan:** None. **D. Maixner:** None. **R. Yadav:** None.

Poster

440. Somatosensory Cortex

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Program#/Poster#: 440.01/II15

Topic: D.09. Tactile/Somatosensory

Title: Beyond columnar organization: Horizontal pathways dominate cortical circuitry

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Abstract: The cerebral cortex is organized into vertical elementary functional units, so called cortical columns. First described by Mountcastle et al., this concept has been most influential for studying the structure, function and computational principles of the mammalian cortex. However, evidence from multiple physiological and functional imaging studies suggested that vertical cortical signal flow is accompanied by horizontal spread. The anatomical basis of such trans-columnar processing remains yet unknown. Using the rodent vibrissal system as a model, we performed single neuron electrophysiological recordings/labeling *in vivo*. By doing so, we determined the complete three dimensional dendrite and axon projection patterns for a large set of excitatory neurons throughout cortical layers 2-6. Surprisingly, we found that horizontal axonal projections (i.e. outside the principal column) exceeded vertical projections. Moreover, horizontal pathways where not random, but reflected organizational principles of the peripheral receptor organs, i.e. the facial whiskers. Combining these striking novel anatomical data with functional recordings of whisker-evoked spiking activity, we integrated the reconstructed neurons into an anatomically detailed model of the rat barrel cortex and reverse engineered the cell-type specific cortical signal flow. We demonstrate that intracortical activation is based upon two excitatory pathways: a horizontal and a vertical one in deep and granular cortical layers, respectively. Our work shows that the current hypothesis of cortical organization needs to be revisited.

Disclosures: R. T. Narayanan: A. Employment/Salary (full or part-time):; Post-Doctoral Fellow. **R. Egger:** None. **H. Mohan:** None. **A. Johnson:** None. **C. de Kock:** None. **M. Oberlaender:** None. **B. Sakmann:** None.

Poster

440. Somatosensory Cortex

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Support: CONACyT grant 176782

Fullbrigth-García Robles fellowship

Title: Cell type-specific subcortical targets of layer 5 projecting neurons in the rat vibrissal cortex

Authors: *G. ROJAS-PILONI[1], M. GUEST[2], A. JOHNSON[2], R. EGGER[3], R. NARAYANAN[3], D. UDVARY[3], B. SAKMANN[2], M. OBERLAENDER[3,2]
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Abstract: Layer 5 pyramidal neurons provide the mayor output of the cortex and consequently likely to be important modulators of sensory and motor processes. This subcortical layer 5 projecting neurons are morphologically and physiologically heterogeneous and project to distinct intracortical and subcortical targets. However, since most of physiological and morphological studies of layer 5 pyramidal neurons have been carried out in unidentified cells, little is known about morphological characteristics related to subcortical projection site. This is important to understand the specificity at single cell level between structural and functional properties *in vivo* in the mammalian brain. Here, we use retrograde neuronal tracing to analyze the distribution of different populations of subcortical projecting neurons in the rat barrel field somatosensory cortex (BFSI). Additionally, we combine retrograde neuronal tracing with whole cell and juxtacellular recordings in order to fill and reconstruct 3D patterns of functional identified neurons and distinguish special morphological characteristics of each cell type. In this way, we provide unprecedented insight into cell type-specific structural and sensory-evoked functional properties of long-range projection neurons in layer 5 of rat vibrissal cortex.

Disclosures: G. Rojas-Piloni: None. **M. Guest:** None. **A. Johnson:** None. **R. Egger:** None. **R. Narayanan:** None. **D. Udvary:** None. **B. Sakmann:** None. **M. Oberlaender:** None.

Poster

440. Somatosensory Cortex

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Topic: D.09. Tactile/Somatosensory

Support: BMBF/FKZ 01GQ1002

Title: Buildingbrains-3d: A tool to integrate neuron morphologies into 3d brain reconstructions

Authors: *A. JOHNSON[1], O. ZHOVNIR[1], R. EGGER[2,3], B. SAKMANN[1,4], M. OBERLAENDER[1,2,5]
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Abstract: BuildingBrains-3D (BB3D, buildingbrains-3d.org) is a scalable platform of web-based tools for assimilating and analyzing complete 3D reconstructions of individual neurons with respect to their precise 3D location in the brain. Presently these reconstructions are the basis for determining excitatory and inhibitory cell-types serving as sampling sets for large-scale dense connectivity simulations over macroscopic scales in the rat barrel cortex. BB3D is scalable, however, and provides a stable platform for Neuroscientists from laboratories around the world to integrate their reconstructions into common reference frames (e.g. rat cortex, see Egger et al.) to extract standardized morphological features and to obtain insights into the organizational principles of neural circuits within and across brains, regions and species.

Disclosures: **A. Johnson:** None. **O. Zhovnir:** None. **R. Egger:** None. **B. Sakmann:** None. **M. Oberlaender:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: BMBF/FKZ 01GQ1002

Title: Reverse engineering sensory perception and decision making: Bridging physiology, anatomy and behavior

Authors: *M. OBERLAENDER[1,2], R. EGGER[1], R. T. NARAYANAN[1], D. UDVARY[1], J. M. GUEST[2], A. JOHNSON[2], G. ROJAS-PILONI[2]
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Abstract: Understanding how the brain is able to transform sensory input into decisions is one of the major challenges of systems neuroscience. While recording/imaging during sensory-motor tasks identified neural substrates of sensation and action in various cortical areas, the crucial questions of 1) how these correlates are implemented within the underlying neural networks and 2) how their output triggers decisions, will only be answered when the individual functional measurements are integrated into a coherent model of all task-related circuits. The goal of our research is to use the rodent vibrissal system for building such a model in the context of how a tactile-mediated percept is encoded by the interplay between biophysical, cellular and network mechanisms. Specifically, rodents decide to cross a gap when detecting its far side with a single facial whisker. This suggests that whisker contact with the platform, if synchronized with an internal motor signal, triggers the decision. To test this hypothesis, we will determine all sensory/motor-related local and long-range whisker pathways, measure whisker-evoked responses of these populations and use the data to constrain network simulations of active whisker touch. Using a multidisciplinary approach, combining *in vivo* electrophysiology, virus injections, custom imaging/reconstruction tools and Monte Carlo simulations, our reverse engineering strategy will provide unmatched mechanistic insight to perceptual decision making and will function as a show case - generalizable across sensory modalities and species - of how to derive computations that underlie behavior.

Disclosures: M. Oberlaender: None. **R. Egger:** None. **R.T. Narayanan:** None. **D. Udvary:** None. **J.M. Guest:** None. **A. Johnson:** None. **G. Rojas-Piloni:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: BMBF/FKZ 01GQ1002

Title: Axonal IN types in rat's barrel cortex

Authors: *D. UDVARY[1], R. EGGER[1], J. GUEST[2], M. HELMSTAEDTER[3], B. SAKMANN[2], D. FELDMEYER[4], M. OBERLAENDER[1]
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Abstract: Classifying cortical interneurons is a major challenge in contemporary neuroscience due to their great diversity. Interneurons are commonly classified by molecular markers, physiological responses or by morphological and topological features. However, there is little overlap between those properties. Here, we present an approach to classify a large dataset of *in vitro*-labeled interneurons (N=204) across a whole cortical column of the rat's barrel cortex based on their axonal morphology. In contrast to many other classification studies we classified the IN morphologies independent of cytoarchitectonic landmarks since IN soma densities are not related to excitatory soma densities. We find five distinct IN types that are independent of their soma and layer location.

Disclosures: D. Udvary: None. **R. Egger:** None. **M. Oberlaender:** None. **J. Guest:** None. **M. Helmstaedter:** None. **B. Sakmann:** None. **D. Feldmeyer:** None.

Poster

440. Somatosensory Cortex

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Program#/Poster#: 440.06/II20

Topic: D.09. Tactile/Somatosensory

Title: Structural basis of sensory-motor control

Authors: *J. M. GUEST[1], R. EGGER[2], G. ROJAS-PILONI[1], P. STRICK[3], B. SAKMANN[1], M. OBERLAENDER[1,2]
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Abstract: The rodent vibrissal system offers an ideal model for studying sensory-motor pathways of the central nervous system. There has been much consideration given to bring insight to the organization of the whisker sensory pathways in the rodent brain. However, the organization of the vibrissa motor output pathway and the integration of sensory inputs involved in whisker movement are not completely understood. The goal of our research is to use the rodent whisker system to understand the functional architecture of the cortical and sub-cortical areas involved with whisker motor output generation. Combining trans-synaptic virus injections with custom-designed brain-wide imaging and analysis we generate an unbiased map of all vibrissal motor pathways. Wild-type rabies virus is deposited into the intrinsic and extrinsic musculature of the mystacial pad, targeting a single whisker. The virus is then transported in a

time dependent manner throughout the central nervous system via vibrissa motor neurons, located in the lateral area of the facial nucleus that directly innervate the whisker muscles. This technique and the unique features of the virus allow us to provide first-time understanding of the structural basis for sensory-motor whisker control.

Disclosures: J.M. Guest: None. **R. Egger:** None. **G. Rojas-Piloni:** None. **P. Strick:** None. **B. Sakmann:** None. **M. Oberlaender:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: Deutsche Forschungsgemeinschaft (German Research Foundation) grants to H.J.L.

Title: Distinct role for the cortical layers revealed by analysis of in-vivo spike-count noise correlations in the rat barrel cortex

Authors: *Y. AMITAI[1], V. REYES-PUERTA[2], J.-J. SUN[2], H. LUHMANN[2], M. SHAMIR[1]
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Abstract: Our concept of the neocortical organization has emphasized the vertical column as the major canonical functional unit, in which neurons share similar sensory-evoked response properties and present relatively high correlation in their firing. In contrast, little is known about the specific roles of the layers in shaping firing patterns and information processing in the neocortex. The cortical layers have been defined mainly by anatomical criteria such as their neuronal types and their afferent and efferent connectivity, and the firing correlations between neurons steeply decline with horizontal distance. Technical difficulties in sampling neurons with sufficient spatial diversity or information on precise location have precluded so far the critical evaluation of these concepts. We used 128-channel “silicon probes” to examine the spike-count noise correlations during spontaneous activity between multiple neurons with identified laminar position and over large horizontal distances, in the barrel cortex of anesthetized rats. These correlations were very stable over periods of tens of minutes. Eigen decomposition of correlation coefficient matrices revealed that the laminar position of a neuron is a significant determinant of these correlations, such that the fluctuations of layer 5B/6 neurons are usually in opposite

direction to those of layers 5A and 4. Moreover, we found that within each experiment, the distribution of the horizontal (within same lamina) correlation coefficients of the spiking, up to a horizontal distance of about 1.5 mm, is practically identical to the distribution of vertical correlation coefficients (between neurons recorded on the same electrode shank). Taken together, these data show that the neuron's laminar position affects its role in cortical processing. Moreover, our analyses reveal that this laminar effect extends over several functional columns. We propose that the influence of the horizontal components within the cortical circuitry exists in a dynamic balance with the influence of the vertical domain, and that this balance is modulated with brain states to shape the network's activity.

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Poster

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Support: PSC-CUNY Award # 65323-00 43

PSC-CUNY Award # 66224-00 44

Title: Morphological characterization of supragranular neurons in the primary somatosensory cortex

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Abstract: The cerebral cortex is essential for cognitive computations, such as the movement of a limb or the detection of objects on the skin surface. The processors of the cortex are individual neurons and the circuits in which they are embedded. It has been shown that specific morphologies are correlated with specific circuit functions. We performed a morphological analysis of layer 2/3 neurons in the barrel cortex of the mouse as an approach to decipher the

neuronal circuit(s) in this region of the primary somatosensory cortex (S1). Our aim was to objectively determine if there are morphological characteristics that can distinguish one group of neurons from another. Individual neurons from the barrel cortex in brain slices from CD-1 mice were labeled non-selectively with DiI using biolistics and reconstructed three-dimensionally from confocal image stacks. Morphological parameters of cell bodies and dendrites of supragranular neurons in the barrel cortex were measured. Cluster analysis following principal component analysis of the morphological parameters revealed distinct groups of neurons. In order to assign "functionality" to the groups *in vivo* injections of fluorescent beads in the ipsilateral M1 were used to label S1 neurons that projected to M1 (a known target of supragranular barrel cortex neurons). Acute sections from these animals were processed with biolistics as described above. These experiments reveal the distribution of M1-projecting supragranular layers in the overall classification dendrogram and show the correlation between anatomical classes of neurons and specific role(s) within the cortical circuit. The present results further support the hypothesis that neurons involved in specific anatomical pathways have unique morphological properties.

Disclosures: S. Sosnowik: None. **J.C. Brumberg:** A. Employment/Salary (full or part-time):; Professor Queens College and the The Graduate Center, CUNY. **C.H. Tse:** None. **A. Tsimounis:** A. Employment/Salary (full or part-time):; Assistant Professor Queensborough Community College.

Poster

440. Somatosensory Cortex

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Support: NIH grant RO1 NS061963

Title: Selective connectivity of corticothalamic neurons in layer 6 of mouse motor cortex with thalamocortical and cortical projection neurons

Authors: *N. YAMAWAKI, G. M. G. SHEPHERD
Physiol., Northwestern Univ., Chicago, IL

Abstract: Corticothalamic (CT) neurons in layer 6 of motor cortex project axons to thalamus. We investigated the synaptic connectivity between CT neurons and pyramidal-tract (PT),

intratelencephalic (IT), and thalamocortical (TC) neurons using an optogenetic-electrophysiological approach. Channelrhodopsin-2 was selectively expressed in projection neurons by viral transfection, and inert fluorescent retrograde tracers were used to label specific projection neurons for targeted whole-cell recording in brain slices. We found that within-class (recurrent, CT→CT) connectivity was strong, but across-class connectivity was selective: CT and IT neurons were reciprocally connected in a layer-dependent manner but CT and PT neurons are mostly unconnected in either direction. Disynaptic inhibitory inputs mostly paralleled the excitatory connectivity, scaling with the amplitude of excitation. Interestingly, monosynaptic TC input was robust onto PT and IT neurons but weak to undetectable onto CT neurons; however, CT neurons robustly innervated TC neurons in thalamus. These results reveal how CT neurons are incorporated into the hierarchically organized local and long-range circuits of motor cortex and motor thalamus.

Disclosures: N. Yamawaki: None. **G.M.G. Shepherd:** None.

Poster

440. Somatosensory Cortex

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Support: NIH F32 NS 084768

NINDS R01 N5069679

Title: Optogenetic dissection of the contribution of direct thalamic inputs to layer 5 in somatosensory cortex

Authors: *Y. HONG, C. O. LACEFIELD, R. M. BRUNO
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Abstract: Recent studies have suggested that pyramidal neurons in cortical layer 5 (L5) can be strongly driven by sparse thalamocortical input, rather than intracortical pathways. Thalamic afferents mainly innervate cortical layer 4 (L4), where cortical processing has conventionally been thought to begin. Information is thought to flow along a hierarchical series of connections from L4 tosuperficial L2/3, to the output cells in L5/6. However, thalamocortical axons give off sparse collaterals elsewhere, notably at the L5/L6 border. Mechanisms exist that could render

sparse projections, often ignored in the study of circuits, highly effective in spreading excitatory activity. This would suggest a more complex scheme of sensory information processing than previously thought, by which information relayed by thalamus reaches cortical output neurons via direct parallel pathways. Using optogenetics, electrophysiology, and behavioral paradigms, we demonstrate that in the mouse somatosensory system, direct thalamocortical inputs can strongly and directly drive L5 output neurons without L4 activity and assess how the direct and indirect pathways to L5 may affect sensory behaviors.

Disclosures: Y. Hong: None. **C.O. Lacefield:** None. **R.M. Bruno:** None.

Poster

440. Somatosensory Cortex

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Topic: D.09. Tactile/Somatosensory

Support: NINDS R01 NS069679

Title: Behavioral correlates of apical dendritic activity in Layer 5 pyramidal neurons of the mouse barrel cortex

Authors: *C. LACEFIELD[1], E. PNEVMATIKAKIS[2], L. PANINSKI[2], R. BRUNO[1]
[1]Neurosci., Columbia Univ., NEW YORK, NY; [2]Statistics, Columbia Univ., New York, NY

Abstract: In the rodent vibrissal primary somatosensory "barrel" cortex, Layer 5 pyramidal neurons send apical dendrites to the surface of the cortex in Layer 1, where they receive excitatory synaptic contacts from the vibrissal motor cortex (vM1), thalamic medial posterior nucleus (PoM), and secondary somatosensory cortex (S2), as well as a host of contacts from diverse inhibitory interneurons and neuromodulatory nuclei. These connections have the potential to influence the processing of whisker sensory information within the cortical circuit in the context of information about self-generated motion, higher-order features of a stimulus, behavioral state, or the relevance of a sensory stimulus to behavior. To elucidate impact of these connections onto Layer 1 apical tufts during behavior, we imaged dendritic calcium activity in a broad population of dendrites from Layer 5 pyramidal neurons in the mouse barrel cortex using two-photon imaging of the genetically encoded fluorescent calcium indicator GCaMP6 while mice performed a whisker-based head-fixed operant task. We found that as a whole, the population of apical tuft dendrites contains information about a wide variety of behavioral

parameters not directly related to whisker touch, from self-generated motion to behavioral outcomes of trials. Furthermore, the activity of single dendrites correlates with subsets of these behavioral parameters. We analyzed how these factors relate to dendritic activity while varying experimental parameters in order to dissociate the influences of individual behavioral factors on dendritic activity. We also examined the stability of encoding by single dendrites individually and among the entire population of dendrites as animals learned the task. To further analyze whether these correlations arise de novo at the level of the apical dendrites, we compared behavioral correlations with the activity of neurons in other cortical layers, namely in Layers 2, 3, and 4. Finally, in order to see how dendritic activity relates to neuronal output, we compared activity in L5 somata with their apical tufts. We conclude that apical dendrites convey context-specific information that influences the processing of sensory information from the periphery. Such a role may aid in the behavior-specific modulation of sensory processing, for instance by gating the communication of Layer 5 neurons with downstream brain areas during sensory behaviors or gating the plasticity of synapses onto these neurons. Finding a precise role for apical dendritic inputs in sensory encoding is key to understanding the integrative properties of the cortical microcircuit as a whole.

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Poster

440. Somatosensory Cortex

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Support: CIHR Grant MOP-12675

Human Frontier Science Program

Title: Wide field functional imaging reveals network boundaries and symmetric transformation operated between areas of mouse cortex

Authors: ***M. P. VANNI**, M. MOHSENVAND, T. H. MURPHY
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Abstract: Strong reciprocal connections exist between the primary (S1) and secondary somatosensory cortex (S2) and primary motor cortex (area M1) within and between both hemispheres. These areas are organized in somatotopic maps of the body. In this study, the topology of functional connections between these areas was explored by using wide field calcium imaging in Emx1-cre X Rosa26-GCaMP3 mice expressing the genetically encoded calcium indicator GCaMP3 in excitatory neurons. Wide field green epifluorescence imaging measured cortical calcium responses through chronic windows or acute craniotomies. Light isoflurane anesthesia produced a relatively constant level of spontaneous activity to establish connectivity relations between arbitrary cortical points using a "seed pixel" approach. During long sequences of spontaneous activity calcium signals recorded of each location of the area S1 were correlated with localized activity in region of homotopic contralateral area S1, ipsilateral area S2 and bilateral areas of M1. Comparably, activity within each location of area S2 was correlated with activity localized in ipsilateral area S1 and M1. Activity within each location of area M1 was correlated with localized activity in region of homotopic contralateral area M1, bilateral areas of S1, and ipsilateral area S2. These long range connections revealed during spontaneous activity were confirmed by intracortical electrical microstimulation. By combining these topologic maps, we observed 3 distinct planes of cortical mirror symmetry. Mirroring occurred at the midline, M1 and S1 boundary, and the S1 and S2 border. These results are consistent with this idea that connections between areas of the somatomotor cortex link similar somatotopic regions and that these maps are reflected across the cortex and hemispheres as mirror images of each other that flipped in orientation and scaled in size. We anticipate that calcium imaging of functional connections using spontaneous activity will enable longitudinal studies during plasticity paradigms or after models of CNS disease such as stroke where the weighting within these connectivity maps could be altered.

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Poster

440. Somatosensory Cortex

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Support: AASDAP

FINEP

INCT Incemaq

FAPERN

CAPES

CNPq

Title: Distributed representation of tactile information streams in prefrontal cortex

Authors: *M. I. SILVA[1], R. C. MOIOLI[1], C. S. DEOLINDO[1], E. MORYA[1], A. C. B. KUNICKI[1], M. A. L. NICOLELIS[1,2,3,4,5]
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Abstract: The medial prefrontal cortex (mPFC) receives multiples afferents from sensory and association areas and has influence over a wide region of the nervous system. Due to its integrative role, the mPFC has been associated with decision making, working memory, planning, and reasoning, all functions which depend on the integration of a vast network of signals. We propose that the mPFC would also be involved in the active tactile discrimination. To test our hypothesis, we recorded single and multi-unit activity in mPFC in Long-Evans rats, discriminating between a wide or a narrow aperture using only their large mystacial vibrissae, to receive a water reward. A total of 141 single and multi-units were recorded in 12 behavioral sessions. We compared the average firing rate of mPFC neurons before the moment the rat broke the photo beam at the discrimination bars and while the whiskers contacted the bars. Firing rate change responses were classified as excitatory or inhibitory. Statistically significant modulation of firing rate was found in 68.62% of the mPFC neurons tested. Anticipatory firing modulations were observed in 43.43% of the mPFC units. The magnitude of anticipatory firing in mPFC was 2.19 ± 0.12 (mean ± SD) spikes per trial, and its duration was 181.42 ± 14.51 ms (mean ± SD). Our results support the existence of highly correlated patterns of anticipatory activity across the mPFC during tactile discrimination and suggest that this behavior depends on more broadly distributed cortical activation.

Disclosures: **M.I. Silva:** None. **R.C. Moioli:** None. **C.S. Deolindo:** None. **E. Morya:** None. **A.C.B. Kunicki:** None. **M.A.L. Nicolelis:** None.

Poster

440. Somatosensory Cortex

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Support: NIH Grant R01EY022987-01

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Title: Functional topography and tuning properties of neurons in the S1 whisker representation of the short-tailed opossum, Monodelphis domestica

Authors: *D. L. RAMAMURTHY[1], A. G. GORDON[2], L. A. KRUBITZER[3]
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Abstract: Short-tailed opossums are marsupials that shared their last common ancestor with placental mammals over 130 million years ago. The early radiation of marsupials makes them an important order of mammals to study; accumulating evidence indicates that some species within this group, such as Monodelphis domestica, bear a close resemblance to the first mammals in terms of brain and body morphology. Thus, understanding the neocortical organization of these animals could provide insight into the evolution of complex cortical circuitry from earlier, simpler forms. Short-tailed opossums exhibit whisker-mediated active touch sensing during exploration through whisking, an energy-consuming, rapid movement of the facial macrovibrissae that requires specialized musculature. Comparative studies of vibrissal function and musculature suggest that early therian mammals possessed the capability to actively control the position of their whiskers. Unlike rats and mice which also exhibit whisking behavior, short-tailed opossums lack the presence of modular cytoarchitecture ("barrels") in the primary somatosensory cortex (S1). While it has been known for decades that the presence of whiskers or whisking behavior is not correlated with the presence of anatomical barrels in S1, there has been a lack of studies examining the functional organization of the whisker representation in species that do not have a barrel cortex. The goal of our study was to examine in detail the neuronal tuning and topography of the whisker representation in S1 of Monodelphis domestica. Multi-unit activity was recorded from layer 4 of S1 in response to the deflection of individual whiskers using a computer-controlled piezoelectric actuator element, in order to quantitatively measure neuronal response and receptive field characteristics. Recordings reveal spatially tuned neurons with receptive fields that are organized topographically, with dorsal whiskers represented more rostrally in S1, and caudal whiskers represented more medially. These results suggest that though the functional organization of the whisker representation is closely linked to the presence of barrel architecture in the form of a precise spatiotopic map in some mammalian species, whiskers can be efficiently represented at the level of the neocortex even in the absence of anatomical barrels. Understanding the constraints and capabilities of circuits involved in whisker-mediated active touch can help us generalize principles in information processing to

other active touch systems, including touch mediated by exploratory movements of the hands and fingertips in humans.

Disclosures: D.L. Ramamurthy: None. **A.G. Gordon:** None. **L.A. Krubitzer:** None.

Poster

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Title: Neuronal activity patterns of posterior parietal cortical activity during active tactile discrimination task

Authors: *A. C. KUNICKI[1], R. C. MOIOLI[1], C. S. DEOLINDO[1], E. MORYA[1], M. A. L. NICOLELIS[1,2,3,4,5]
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Abstract: Previous studies have investigated the role of primary and secondary sensorimotor cortices during active whisker discrimination task, but overlooked of the posterior parietal cortex (PPC). PPC is located between multiple sensory regions and receives projections from somatosensory, auditory, and visual areas, and may therefore be important for processing multimodal sensory information. Since the trigeminal inputs from whiskers reaches PPC through thalamic and somatosensory areas, we expected that the posterior parietal cortex (PPC) would also be involved in tactile discrimination. To test our hypothesis, we recorded single and multi-

unit activity in PPC in Long-Evans rats discriminating between a wide or a narrow aperture using only their large mystacial vibrissae to receive a water reward. A total of 126 single and multi units were recorded in 6 behavioral sessions. We compared the average firing rate of PPC neurons before the moment the rat broke the photo beam at the discrimination bars (anticipatory period: $-0.5<t<0$ s) and while the whiskers contacted the bars (discriminatory period: 0.30 s). Firing rate change responses were classified as excitatory or inhibitory. Statistically significant modulation of firing rates were found in 60.21% of the PPC neurons tested. Anticipatory firing modulations were observed in 38.18% of the PPC units. The magnitude of anticipatory firing in PPC was 2.59 ± 0.1 spikes per trial, and its duration was 183.4 ± 11.72 ms. Our results suggest that PPC neurons are involved in active tactile information, fully supporting the hypothesis that this behavior relies on widely distributed cortical activation.

Disclosures: **A.C. Kunicki:** None. **R.C. Moioli:** None. **C.S. Deolindo:** None. **E. Morya:** None. **M.A.L. Nicolelis:** None.

Poster

440. Somatosensory Cortex

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 440.16/II30

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant F30 MH097425

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Title: Mapping the connectivity of a cortical chandelier cell module

Authors: *J. TUCCIARONE[1,2], J. LU[1], J. HUANG[1]
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Abstract: In the mammalian neocortex, the delicate balance and functional dynamics of neural circuits are regulated through a rich repertoire of inhibitory control mechanisms mediated by diverse classes of GABAergic interneurons. Chandelier cells (ChCs) are a highly unique class of fast spiking interneuron that specifically control pyramidal neurons (PyN) at axon initial segments (AIS)- the site of action potential generation. Since each ChC innervates hundreds of PyNs, ChCs are poised to powerfully regulate PyN ensemble firing. Using genetic fate mapping

with the Nkx2.1-CreER mouse line, we previously discovered the developmental origin and laminar organization of ChCs. The striking stereotypy and specificity of ChCs may define a cortical "connectivity module", but pattern of ChC innervation and recruitment remains to be determined. We have developed a method that allows single ChC labeling and manipulations. Here we combine single cell reconstruction, rabies tracing, multi-patch recording, and optogenetics to define a layer 2 "ChC module" in mouse somatosensory and frontal cortex. The axon arbor of single L2 ChC is largely restricted in L2 and innervates 300±50 AIS as estimated by the number of ChC "cartridges," indicating dense local control of PyNs. Paired recording in L2 revealed that while ChCsPyN connection is prevalent, while PyNChC connection is sparse. This predominant one-way ChCsPyN connectivity is in sharp contrast to the well-known reciprocal pattern between fast-spiking basket cells and PyNs, suggesting a distinct connectivity module. Monosynaptic rabies tracing revealed that L2 ChCs mainly receive inputs from L3 and subpopulations of L5 pyramidal cells, suggesting largely translaminar local recruitment. ChCs also receive inhibitory inputs from parvalbumin and somatostatin interneurons. In addition, long-range inputs originate from the ventral, medial and posterior thalamic nuclei, several ipsilateral and contralateral cortical regions, and subcortical neuromodulatory nuclei such as the diagonal band nucleus. Channel Rhodopsin Assisted Circuit Mapping (CRACM) validated several of these input sources. Together our studies begin to define a ChC module and its local and long-range inputs. These connectivity maps provide a entry point to explore the role of ChCs in cortical processing.

Disclosures: J. Tucciarone: None. **J. Lu:** None. **J. Huang:** None.

Poster

440. Somatosensory Cortex

Location: Halls A-C

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Program#/Poster#: 440.17/JJ1

Topic: D.09. Tactile/Somatosensory

Title: Spatial organization of neurons coding for complex multi-whisker features in S1bf

Authors: *J.-F. LEGER[1], L. ESTEBANEZ[2], J. BERTHERAT[1], D. E. SHULZ[3], L. BOURDIEU[1]
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Abstract: During exploration of the environment, rats contact objects with multiple whiskers in a complex spatio-temporal pattern. Recent electrophysiological experiments (Estebanez et al., Nat. Neurosc. 2012) have demonstrated using multi-whisker sensory stimulations the existence in layers 4 and 5 of the barrel cortex of "global" neurons that encode correlated deflections of all whiskers ("correlated stimulation") and "local" neurons that detect the deflection of their principal whisker ("uncorrelated stimulation") and that can exhibit an enhanced response to angular contrast between the principal and surrounding whiskers ("anti-correlated stimulation"). We used two-photon fluorescence microscopy (TPFM) to explore the existence and the spatial organization of similar tuning to multi-whisker stimuli in the layer 2/3 of the barrel cortex, where connections between neurons from different barrels might favor multi-whisker tuning. A 25-whisker stimulator that allows the stimulation of each whisker in all directions (2D) and with a 1kHz bandwidth (Jacob et al., 2010) was combined with TPFM to record optically the sensory-evoked cortical activity. By combining functional imaging in the presence of uncorrelated, correlated and anti-correlated stimulations and post-mortem histology with 50µm resolution, we demonstrate the existence in layer 2/3 of different categories of cells tuned to multi-whisker stimuli and that partially correspond to what was described in deeper layers. Moreover the different categories are spatially segregated in this layer. This spatial segregation is pointing to the possibility that different neural networks in the upper layers of the barrel cortex sustain different functional roles in sensory processing and are put into play by different input statistics.

Disclosures: J. Leger: None. **L. Estebanez:** None. **J. Bertherat:** None. **D.E. Shulz:** None. **L. Bourdieu:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS026143

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Kavli Institute for Neuroscience

Title: Motor cortex ensemble activities during goal-directed behavior

Authors: *E. ZAGHA, X. GE, D. A. MCCORMICK
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Abstract: Neocortex is an essential mediator of goal-directed behaviors. However, we still lack a comprehensive understanding of the mechanisms underlying neocortical function. We developed a simple sensory detection task in mice in order to address the following questions: what is the operating mode of cortex during goal-directed behavior? How are task parameters represented by neural ensembles? What are the neural mechanisms underlying discrete behavioral transitions? The behavioral paradigm is a cross-modal cued sensory detection task, in which mice are trained to withhold behavior until after a target stimulus. We find that motor cortex neurons are persistently activated during the task, with coherent slow, oscillatory activity variably expressed only during the inter-trial intervals and/or when mice are disengaged from the task. We find that the neural representations of task parameters are highly redundant, with most motor cortex neurons contributing to delay (between cue and target) or post-target activity. We also observe robust and rapid transitions in ensemble activity immediately following the target, which predicts reaction times on a trial-by-trial basis. These data are consistent with a model of motor cortex as occupying discrete states of ensemble activity, with rapid transitions between stable states reflecting anticipatory or motor signaling.

Disclosures: E. Zagha: None. **X. Ge:** None. **D.A. McCormick:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: Spain Ministry of Economy and Competitiveness grant BFU2011-23049 (co-funded by FEDER)

Valencia Regional Government grant PROMETEO/2011/086

Title: Coding of sensory sequences in the barrel cortex: Time integration versus sensitivity to instantaneous parameters

Authors: *M. MARAVALL, A. L. ALBARRACÍN, M. MOLANO-MAZÓN, A. PITAS, M. R. BALE
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Abstract: Making sense of the world requires integration of sensory patterns and sequences over time. For example, recognizing a song or deciphering speech requires the identification and classification of a stream of vibrations. Such sequence selectivity is central to many behaviors and is a hallmark of cortical output, yet surprisingly little is known about the sites and mechanisms of temporal integration and sequence representation. Here we begin to map cortical signatures of sequence selectivity in the whisker system. We performed juxtacellular recordings from barrel cortex (BC) in anesthetized mice. Controlled whisker stimulation was applied with a piezoelectric actuator and consisted of sequences of deflections at random intervals. We compared the information carried by individual neurons about the latest stimulus interval (reflecting sensitivity to instantaneous frequency) and about earlier intervals (reflecting integration over time). Neurons throughout BC layers carried substantially more information about the latest single intervals than about earlier intervals or interval sequences; a subset of supragranular neurons displayed the strongest integration. The most responsive neurons, located in middle layers, integrated particularly weakly over time. These results support the notion that primary sensory cortex is principally an encoder of current stimulation parameters. To assess sequence discrimination behavior rigorously and to validate our results in awake animals, we have developed a task where mice detect and discriminate specific temporal patterns of stimulation using a head-fixed, go/no-go design.

Disclosures: M. Maravall: None. **A.L. Albarracín:** None. **M. Molano-Mazón:** None. **A. Pitas:** None. **M.R. Bale:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

Location: Halls A-C

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Topic: D.09. Tactile/Somatosensory

Support: Israel Science Foundation

Title: Whisker mediated tactile detection in the rodent somatosensory system

Authors: *P. KURUPPATH, R. AZOUZ
Ben-Gurion Univ., Beer Sheva, Israel

Abstract: Rodents use their whiskers to gather information about their immediate environment. When the rats sweep their whiskers against an object or different surfaces of their nearby surroundings, mechanoreceptors located in the whisker follicles detect the tactile signals as whisker vibrations and transduce this whisker vibration signals into action potential. Which of the physical parameters in the tactile signals are extracted by the rodent somatosensory system for detection is unknown. Here, we employed a psychophysical task in head-fixed rats to identify the physical stimulus features that influence tactile detection. To identify the stimulus features involved in the detection, we employed Go/NoGo task in which rats are required to detect the stimulus and respond by licking on the water spout for the reward. Our results show that rat's detectability changes with the changes in the stimulus frequency, variance, velocity and duration. However, we find that detection performance based on extraction of transient kinematic events is far superior to that based on the above parameters.

Disclosures: P. Kuruppath: None. **R. Azouz:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Program#/Poster#: 441.04/JJ5

Topic: D.09. Tactile/Somatosensory

Support: ISF Grant

Title: Population coding in the trigeminal system

Authors: *E. GUGIG, R. AZOUZ
Ben Gurion Univ. of the Negev, Beer Sheva, Israel

Abstract: Rodents use their whiskers extensively to explore their immediate surroundings. Each whisker's follicle consists of few hundreds of mechanoreceptors which signal several aspects of whiskers' movements, and transmit accurate and reliable tactile information downstream to the rest of the brain. As most of the information available to the somatosensory system originates in whiskers' primary afferents, it is essential to understand the transformation of whisker motion into neuronal activity of a population of mechanoreceptors. However, since these are not aligned

somatotopically, deciphering the coding of an assembly of primary afferents is challenging. To address this issue we recorded responses from a "virtual population" of Trigeminal Ganglion neurons to two-dimensional frozen white noise stimulus. We mimicked simultaneous recording of the population by maintaining the stimulator position untouched between the different neuronal recordings, while recording from multiple single primary afferents. We find that these neurons can be divided into three distinct subtypes, each carry information about different kinetic feature of the stimulus. Our results show that when stimulated with the same orientation, different neurons exhibit different responses. Existence of three distinct subtypes together with these findings emphasis the need to study the tactile information that emerges from a population of primary afferents. Specifically, we are interested to examine how the different mechanoreceptors contribute together as population to the representation of the overall information valid to the animal. We would address the question whether the information from the population is redundant, synergistic or even independent.

Disclosures: E. Gugig: None. **R. Azouz:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Title: Context-dependent information decoding of sensory-evoked responses

Authors: *H. J. ZHENG[1], C. J. SHEPHARD[1], B. J. HE[2], G. B. STANLEY[1]
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Abstract: The cortical response to an external sensory stimulus is embedded in the spontaneous activity in the cortex that is constantly ongoing and dynamic, resulting in a highly variable representation of the sensory input that yet can produce relatively consistent stimulus perception. To characterize the sensory-evoked response, the traditional approach has been to model the variability as the linear superposition of a stereotypical, trial-averaged response and the constantly changing spontaneous activity. However, without accounting for the potential interaction between the ongoing spontaneous activity and the sensory-evoked activity on single-trial basis, the trial-averaged response cannot provide a complete picture of the dynamic processes in the brain. To model the potential interaction between the spontaneous activity and sensory-evoked response, we recorded spontaneous and sensory-evoked brain activity with simultaneous local field potential (LFP) and voltage-sensitive dye (VSD) in the same cortical column of the primary somatosensory cortex in the anesthetized rat. As a model of information decoding, we classified the intensity of the sensory stimulus from the perspective of an ideal observer of the cortex on a single-trial basis. We hypothesize that, if the spontaneous activity can at least partially account for the variability in the evoked responses, then observing spontaneous activity in addition to sensory-evoked activity would enhance the stimulus intensity classification performance. Our preliminary results show that LFP and VSD recordings are correlated on single-trial basis and both exhibit a negative correlation between pre-stimulus activity and sensory-evoked activity, as has been previously demonstrated in the awake human brain (He, 2013). By utilizing both pre-stimulus activity and the sensory-evoked response, the two-dimensional response distributions of two distinct stimulus intensities are less overlapped than the one-dimensional response distributions of only the sensory-evoked response, resulting in an enhanced stimulus intensity classification performance. Taken together, our preliminary results suggest a role for the ongoing spontaneous activity in information processing in sensory pathways, potentially reflecting a state-dependent framework of neural encoding. Reference: He, Biyu J. "Spontaneous and task-evoked brain activity negatively interact."The Journal of Neuroscience 33.11 (2013): 4672-4682.

Disclosures: **H.J. Zheng:** None. **C.J. Shephard:** None. **B.J. He:** None. **G.B. Stanley:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Support: Swiss National Science Foundation

Human Frontier Science Program

SystemsX.ch

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European Research Council

Title: Parvalbumin-expressing GABAergic neurons gate sensory perception in mouse barrel cortex

Authors: *S. B. SACHIDHANANDAM[1,2], C. C. H. PETERSEN[2]
[1]Brain Res. Institute, Univ. of Zurich, Zurich, Switzerland; [2]Brain Mind Institute, Lab. of Sensory Processing, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: The neocortex has a diversity of GABAergic neurons that display an equally diverse range of activity in awake animals. However, they can be classified into groups based on their expression of largely non-overlapping molecular markers. We took advantage of this and examined action potential firing of vasoactive intestinal peptide-expressing (VIP), somatostatin-expressing (Sst) and parvalbumin-expressing (PV) GABAergic neurons during goal directed behavior. Mice were trained to detect single brief whisker stimuli and to report perceived stimuli by licking to obtain a reward (Sachidhanandam et al, 2013). Under visual control offered by two-photon microscopy, juxtasomal recordings were then targeted to genetically-defined types of GABAergic neurons in layer 2/3 barrel cortex. We observed that VIPneurons increase their firing rates following whisker deflection to comparable levels in both hit and miss trials. Conversely Sst neurons showed an initial brief increase in firing, followed by a decrease, after whisker deflection in both hit and miss trials. However, Sst neurons displayed elevated firing during licking in hit trials. PV neurons on the other hand reduced their firing rates during the late sensory period in hit trials before licking onset compared to misses, hence showing task outcome related differences in firing activity. This correlated with an increase in firing rates of excitatory cells during the late phase, prior to licking. Subsequent optogenetic inhibition of PV neurons during the late phase resulted in an increase in performance. Our data suggest that PV neurons gate the late sensory response in the whisker detection task that drives the subjective percept as reported through licking.

Disclosures: S.B. Sachidhanandam: None. **C.C.H. Petersen:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: DSRG, CUNY.

Title: The neural correlates of motion within the rodent somatosensory cortex: The design and control of a multi-whisker stimulator

Authors: ***A. A. BAJNATH**[1], V. LIU[2], I. LAX[2], J. C. BRUMBERG[2,3]
[1]Psychology, Queens Col. & The Grad. Center, CUNY, FLUSHING, NY; [2]Psychology, Queens College, City Univ. of New York, New York, NY; [3]Biol. & Psychology, The Grad. Center, CUNY., New York, NY

Abstract: The ability to detect sensory stimuli plays a critical role in the survival of any animal. For the nocturnal rodent, the task of detecting tactile sensory stimuli in order to guide navigation through its environment is accomplished by an array of whiskers on the animal's mystacial pad. This entire array is mapped topographically within the somatosensory cortex (S1) of the rodent brain and forms a sensory map. Within S1, there are clusters of neurons known as 'barrels' which correspond topographically to the spatial organization of whiskers in a one-to-one fashion. To investigate how the rodent barrel cortex encodes features of a moving stimulus across the animal's whisker pad, we utilized a novel programmable whisker stimulator that allows us to present controlled moving stimuli in either the forward or backward direction at different speeds to an anesthetized mouse. Stimuli consisted of a smooth drum and a textured drum of identical diameter with repeating grooves ~3mm wide. Video recordings indicate that many of the caudal (Arcs 1-3) whiskers are engaged by our stimulus. When the stimuli were presented, both drums drove cortical neurons to fire; responses to the textured stimulus resulted in increased neural firing when compared to the responses of the smooth stimulus. There is adaptation throughout the duration of the stimulus in both the smooth and textured conditions and rates of adaptation varied with stimulus speed. Barrel neurons displayed preferences for specific speeds, texture and motion in one direction versus the other. Our results suggest that mouse barrel cortex processes motion information at the early stages of cortical processing.

Disclosures: **A.A. Bajnath:** A. Employment/Salary (full or part-time):; Graduate Assistant, CUNY.. **V. Liu:** None. **I. Lax:** None. **J.C. Brumberg:** A. Employment/Salary (full or part-time):; Professor, Queens College.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: NIH R01NS 039050-13 (RSE)

NSF Career Award (YC)

Title: Voltage-sensitive dye optical imaging of cortical whisker frequency selectivity

Authors: *V. TSYTSAREV[1], E. PUMBO[3], Q. TANG[2], C.-W. CHEN[2], Y. CHEN[2], R. S. ERZURUMLU[1]
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Abstract: The mystacial vibrissae (whiskers) of nocturnal rodents act as a high-resolution tactile apparatus that allows the animal to detect the finest details of its environment in the dark. A decade ago, Andermann et al., (Neuron, 2004) reported that whisker-sensitive somatosensory neurons in the trigeminal ganglion and the somatosensory cortex show frequency selectivity to small amplitude stimuli, akin to frequency selectivity of auditory system neurons. Whisker resonance and frequency selectivity in whisker-sensitive neurons might play an important role in fine tactile discrimination. Using *in vivo* voltage-sensitive dye optical imaging (VSDi), we performed functional brain mapping of the mouse somatosensory cortex in response to the mechanical stimulation of a single whisker with different frequencies. Experiments were performed on six B6 mice, 25-30 g body weight, at 6-10 weeks of age using MiCAM-02 system (MiCAM-02, Brain Vision Inc., Japan), using the voltage-sensitive dye RH-1691 (Optical Imaging Ltd, 1.0 mg/ml in ACSF). Multiple steps of data analysis were used to study the spatial and temporal features of the evoked fluorescence in the face representation area of the primary somatosensory cortex ("barrel field".) We derived pseudocolor maps of the neural activity and identified the activated cortical areas by the number of pixels exhibiting a change in fluorescence ($\Delta F/F$) greater than 50% of the maximum change in signal. The differences between responses to the single pulse mechanical stimulation at 100, 200, 333 and 500 Hz frequencies within 60 ms were indicated by a change in the voltage-sensitive dye optical signal. We used the data to chart the time course of the fluorescence signals. We found that whisker mechanical stimulation with different frequencies led to different fluorescence signal in the barrel field: the response of 333 Hz was significantly stronger than the rest of the frequencies. Our results provide further

evidence that the different neurons of the barrel cortex have different frequency preferences. We suggest that frequency selective neurons in the barrel field are characterized by a V-shaped histogram of the stimulation frequency /stimulation intensity similar to auditory cortical neurons that show a V-shaped histogram of sound frequency/sound intensity.

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Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: NIH R01NS48285

Georgia Research Alliance

NSF GRFP

Title: Probabilistic encoding of stimulus features in layer 2/3 of the rodent barrel cortex

Authors: *C. A. GOLLNICK, D. C. MILLARD, R. V. BELLAMKONDA, G. B. STANLEY
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Abstract: The traditional model of sensory processing is a hierarchical pathway where neuronal receptive fields become increasingly complex with each stage of convergent neural input. While this model has proven useful in the study of vision, evidence for hierarchical processing in other sensory modalities, particularly somatosensation has proven elusive. In the absence of feature-selective neurons, it is not clear how stimulus features, such as the direction and speed of stimulus, are encoded. We suggest a probability of activation framework in which specific stimulus attributes are encoded by an array of detectors that respond probabilistically with increasing stimulus intensity. Using the rodent barrel cortex as a model, we first present evidence that individual columns act as independent but unreliable detectors of mechanical stimuli. We record layer 2/3 population responses to whisker deflections with voltage sensitive dyes and systematically vary whisker deflection velocity, a measure of stimulus intensity. An ideal observer performing Bayesian classification of single trials into intensity categories performed poorly and made large errors. As we observed both large response amplitudes to small sub-

threshold deflections as well as small responses to large deflections, we concluded that absolute amplitude of the response does not encode stimulus intensity. Instead, the response reliability increased with stimulus intensity. Individual barrels responded independently to simultaneous whisker deflections suggesting that barrels can act as redundant detectors of mechanical stimuli. Non-linear temporal suppression dynamics could also be explained by probabilistic trial-to-trial response variability. When taken together these data suggest that stimulus features, even stimulus events, were not encoded reliably within a single barrel. We propose a model in which stimulus features such as direction of motion and intensity could be decoded spatially across an array of cortical columns that respond independently, but unreliably, to mechanical stimuli.

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Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant 5R01NS045130-09

Title: Cortical representation of stimulus changes in barrel cortex by temporal sharpening

Authors: *J. VOIGTS[1,2], C. A. DEISTER[2], C. I. MOORE[2]
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Abstract: Hierarchical sensory processing rests on the brain's ability to build sparse and predictive models of sensory data. A central component of predictive models is the computation of a change signal that represents the agreement between the expected and true sensory input. To investigate the cortical implementation of this computation, we recorded spike trains in the somatosensory cortex of awake mice while presenting vibrissa stimuli containing small changes in stimulus size. In contrast to previous studies that found an overall increase in firing rates for big stimulus changes, sensory driven neurons didn't generally increase their firing rates in response to the stimulus deviations. Instead we observed an overall higher spike synchronization elicited by the deviant stimuli. These effects were observed regardless of stimulus identity and amplitude. In a stimulus detection task, deviant stimuli were more detectable at low stimulus

amplitudes. This suggests that temporal encoding of stimulus changes could function as a marker of salience for unexpected stimuli, while the diversity of firing rate changes elicited by the deviant stimuli represents a local cortical error signal that depends on each neurons receptive field properties. To investigate the role of local cortical inhibition in this process, we optogenetically increased cortical inhibition at levels that did not affect baseline detection behaviour performance. The manipulation shifted firing rates to a linear regime where cells represented the current stimulus, rather than the change relative to the previous stimuli. Further, the manipulation selectively negated the behavioural benefit of deviant stimuli. Our findings outline a cortical mechanism that normalizes responses relative to previous stimuli and computes a local cortical change signal.

Disclosures: J. Voigts: None. **C.A. Deister:** None. **C.I. Moore:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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C.I.M. NIH/NINDS R01NS045130

C.I.M. NIH/NINDS R56NS045130-10A1

Title: Maximally informative neurons show high levels of internal correlation in an otherwise decorrelated cortex

Authors: *C. A. DEISTER[1], S. BECHEK[1], R. LICHTIN[1], T. BROWN[1], J. VOIGTS[2], C. MOORE[1]
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Abstract: Correlations in neural activity that occur independent of explicit sensory drive are often viewed as detrimental to coding, because they limit the ability to employ optimal rate-coding schemes. Even weak correlations force larger pools of neurons to be averaged to distinguish shared fluctuations (or noise) from incoming signal. In support of this view, these spike count correlations (r_{sc}; also known as “noise“ correlations) have been found to decrease during active sensory processing and/or with deployment of selective attention. However, other

views of neocortical processing rely on dynamics of cortico-cortical efferents in coordinating small ensembles for representation; a situation seemingly counter to the limitations imposed for efficient coding. Cortex doesn't have to implement an efficient code, but what then is the role of active decorrelation? We address this question we used two-photon microscopy to image the spiking activity of neurons in layers II/III of mouse vibrissal somatosensory cortex (vS1; barrel cortex), expressing the genetically encoded calcium indicator GCaMP6s. Head-fixed mice were trained to report the detection of vibrissa stimuli by licking for a water reward. We focused on trials with stimuli that elicited a detection response in roughly half of the time, and asked if firing rate dynamics could predict detection. Consistent with previous studies, the majority of neurons did not respond to sensory stimulus (NR; ~74%). The majority of neurons that reliably responded to the sensory stimulus could not predict detection (NPr; ~23%), but a small proportion were able (Pr; ~3%). Rate fluctuations in Pr neurons did not simplistically encode motor output, as their rates were indistinguishable from NPr neurons in their ability to predict licking. The response groups differed in their average r_{sc}, which increased with task-predictability (NR = 0.02; NPr = 0.08; Pr = 0.14). NPr neurons (the majority of sensory neurons) showed the effects of decorrelation on successfully detected trials, with the entire r_{sc} distribution shifting to lower values. NPr neurons showed no differences with detection. Perceptually invariant, high correlations among the most informative neurons (the NPr group) are inconsistent with the view that decorrelation is essential to relay sensory information, but it may be a component. We suggest that optimal neocortical representation has the following features: 1) Enhanced firing rate in a maximally informative ensemble; 2) higher correlation and coordination between elements of this ensemble; and 3) a desynchronization of elements with less informative and potentially competing (or distracting) information.

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Poster

441. Somatosensory: Stimulus Feature Neural Coding

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 441.12/JJ13

Topic: D.09. Tactile/Somatosensory

Support: CNRS

AMU

Title: Neuronal signature of the tactile "funneling illusion": Population encoding in S1 cortex

Authors: N. CATZ, Y. ZENNOU-AZOGUI, J. CORBO, *C. A. XERRI
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Abstract: Despite increasing interest in studying the neural correlates of perception, neuronal population coding in somatosensory cortex has received little attention. We investigated the neural substrates of the "funneling illusion" in which simultaneous presentation of brief tactile stimuli at two skin loci produces a single focal sensation at the center of the stimulation pattern even though no physical stimulus is applied at that site. This illusion raises the question of how sensation is encoded in the brain and whether cortical activation matches the actual or perceived stimulation. Previous studies using intrinsic optical imaging and fMRI in monkeys (Chen et al., 2003, 2007) showed that simultaneous stimulation of adjacent digits produced a single activation located between the digit representational zones in area 3b, whereas costimulation of non-adjacent digits produced separate activation spots. We used voltage sensitive dye imaging (VSD) and single-unit recording in anesthetized rats to investigate the spatial-temporal dynamics of cortical integration in S1, in stimulation conditions inducing the funneling illusion. Our VSD data show that, in contrast to Chen's study, costimulation of non-adjacent digits induced a single activation initially occurring within the representation of the intermediate, i.e. perceptually referred, non-stimulated digit. During single-digit stimulation, S1 units displayed a "preferred" response consistent with the topographic map organization and a smaller response to adjacent digits. During costimulation of two non-adjacent digits, units displayed firing rates that tended to mimic that generated by individual stimulation of the intermediate digit as if a real stimulation had been applied on this digit. To determine how tactile stimulus could be read off from the activity of neuronal populations, we used a bayesian decoding procedure. We trained our algorithm with the population responses obtained for single digit stimulation and tested it by "injecting" the population responses recorded in the funneling stimulation conditions. For the co-stimulation of non-adjacent digits, about 60 % of the trials were predicted by the decoder to fit the stimulation of the intermediate digit alone. The decoding procedure reveals that co-stimulation of non-adjacent digits evokes a S1 population response that constitutes a potential substrate of the funneling illusion. In contrast to the traditional view, topographically organized neuronal networks represent not only the peripheral stimulus characteristics, but also contribute to the emergence of contextually defined illusory perception and somatosensory awareness.

Disclosures: **N. Catz:** None. **Y. Zennou-Azogui:** None. **J. Corbo:** None. **C.A. Xerri:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: CIHR

GRSNC (FRQS).

Title: Neuronal correlates of tactile surface roughness in primary somatosensory cortex, S1, of the macaque monkey

Authors: *E.-M. MEFTAH, S. BOURGEON, A. DÉPEAULT, C. E. CHAPMAN
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Abstract: Using manufactured raised-dot surfaces, we have shown that subjective roughness shows a monotonic increase over a wide range of spatial periods (SP, distance centre-to-centre between rows of raised dots), with larger SPs (8.5mm) feeling rougher than smaller SPs (1.5mm). All of the cutaneous mechanoreceptive primary afferents involved in discriminative touch are coactivated when such textures are scanned over the skin but the sensory signals are ambiguous, varying with surface texture, scanning speed & contact force. Despite this, subjective roughness is invariant with 2-fold changes in scanning speed (Meftah et al. 2000), suggesting that the brain is able to extract an invariant representation of roughness from the complex afferent signals. We hypothesized that the discharge of cortical neurones involved in scaling tactile roughness should covary with tactile roughness across a wide range of SPs and be independent of speed. To test this hypothesis, we recorded from 185 neurones in the hand region of S1 cortex (69, 75 and 41 cells in areas 3b, 1, and 2 respectively) of 4 awake monkeys. All cells had a cutaneous receptive field on the tips of digits 3 and/or 4. Raised-dot surfaces (longitudinal SP varying from 1.5-8.5mm) were moved passively under the fingertips (D34) at different speeds, 40-115 mm/s. All cells were modulated during surface scanning. Cells were categorized as sensitive to texture-only (49%), texture + speed (31%) or speed-only (8%). Of particular interest here were the cells categorized as texture-only. These were found in all 3 areas: 48 in area 3b, 34 in area 1 and 9 in area 2. Texture-only cells were especially concentrated in area 3b (79% of the sample) as compared to only 45 and 22% of the samples in areas 1 and 2. Consistent with a role in tactile roughness scaling, 23/91 texture-only cells showed a graded increase in discharge with increasing SP independent of speed. The majority of these were located in area 3b (15). Overall, these cells made up only 16% of the texture-sensitive neurons, consistent with a select group of S1 cells coding roughness unambiguously (sparse coding). The remaining texture-only cells were categorized as either “non graded” (60) or variable (8, including 2 with an inverted-U profile). The discharge of non graded cells plateaued over a portion of the range of SPs, with graded changes in discharge being restricted to a small range of SPs (43%, ≤ 3mm). Such cells could play a role in discriminating small differences in SP. Non

graded cells were found in all 3 areas, but were again concentrated in area 3b (32/60). Thus area 3b appears to play a key role in coding tactile roughness independent of speed.

Disclosures: **E. Meftah:** None. **S. Bourgeon:** None. **A. Dépeault:** None. **C.E. Chapman:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Support: MEXT KAKENHI Grant Number 26440176

Title: Estimation of neuronal assembly encoding directional information by decoding method

Authors: ***M. SOMEYA**[1], H. OGAWA[2]
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Abstract: Population coding in which specific information is encoded as population activities by neuronal assembly is widely adopted in the nervous system. The schemes of population coding depend largely on assembly size and response characteristics of constituent neurons, but neuronal assemblies encoding specific types of sensory information have not yet been identified. To estimate the size of a neuronal assembly encoding specific sensory information using the decoding approach, simultaneous recording of the total activity from all candidate neurons is required. However, this is technically difficult because of the variety and huge number of neurons in the mammalian brain. The insect nervous system composed of fewer neurons allows us to resolve this problem. In this study, we used the cricket cercal system, which has been well-studied in neural coding and circuits. The cercal system mediates the detection, localization, and identification of surrounding air currents. In the cercal system, sensory information of airflow is processed by local circuit within the terminal abdominal ganglion and conveyed by identified ascending projection neurons to thoracic and cephalic ganglia. Based on total activity recorded from the ascending projection neurons by a pair of suction electrodes, a decoding algorithm was used to estimate the size of a neuronal assembly encoding directional information of air current stimuli. In the result, a neuronal assembly comprising 14 units encoded adequate information on stimulus direction, which was decoded with the highest accuracy in the declining phase of transient firing. Preferred direction of the neuronal units composing the estimated neuronal

assembly was biased toward the postero- and ipsilateral side to their axon. Further, the accuracy of the assembly readout was consistent with variation in walking orientation of the air-current-elicited behavior. The results suggest that directional information could be encoded in a simple schema by a neuronal assembly consisting of relatively few components that have unique and biased response properties in the cricket nervous system.

Disclosures: **M. Someya:** None. **H. Ogawa:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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FP7 Biotact EU

ERC Neuro-behavior

Title: Enhancement and modelling of spike timing reliability in-vivo using noise evoked by juxtacellular stimulation

Authors: ***G. DORON**, J. DOOSE, M. BRECHT, B. LINDNER
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Abstract: We have recently shown that the juxtacellular nanostimulation technique can be used to parametrically control spike frequency and number in identified single cortical neurons *in vivo* (Houweling et al., 2010). Specifically, we found that spike number in barrel cortex neurons varies linearly both with stimulus intensity and stimulus duration, using step current injections. However, using this method we were not able so far to achieve spike timing reliability. Here we are extending these findings and show that driving pyramidal cells in anesthetized rat vibrissal motor cortex with fluctuating stimuli (frozen bandpass-limited white noise) results in increased spike timing reliability. Specifically, we report that parametrically increasing the nanostimulation noise level results in increased spike train synchronization. In addition, it results in increased cross spectra between the stimulus and the spike trains, which leads to increased coherence and change in the coherence shape. We further explore how well the spike train in response to this stimulus can be captured by an exponential integrate-and-fire neuron (Fourcaud-Trocme et al.,

2003), a simple model that has been successfully applied for reproducing spike times of pyramidal cells under noisy current stimulation *in vitro* (Badel et al., 2008). In contrast to the latter situation, our model also includes an appreciable amount of intrinsic noise, accounting for fluctuating input from the surrounding network. Nanostimulation therefore permits enhanced control of spike timing in single cortical neurons and therefore holds great potential for elucidating how spike timing reliability in single neurons may contribute to behaviour.

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Poster

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Topic: D.09. Tactile/Somatosensory

Support: NSF Grant IOS-1150209

Title: Spatial variation of simulated slowly adapting type 1 afferent responses to embossed dot patterns predicts perceived roughness

Authors: *J. GOODMAN, JR, J. LIEBER, H. SAAL, S. BENSMAIA
Univ. of Chicago, Chicago, IL

Abstract: The perception of embossed dot patterns is dominated by the responses these textures evoke in slowly adapting type 1 (SA1) afferents. In particular, perceived roughness of such course textures is thought to be encoded by the spatial variation of SA1 responses. However, manipulations of dot height have yielded roughness judgments that call this neural code into question by showing that the psychophysical function relating perceived roughness to inter-dot spacing is modulated by the height of the dots. In the present study, we wished to determine whether a spatial variation code could account for roughness judgments of embossed dot patterns across inter-dot spacings and dot heights. To this end, we implemented a continuum mechanical model of the skin to simulate the spatial distribution of loads applied by dot patterns. We then computed the maximum compressive strain - thought to drive SA1 responses - at a fixed depth in the skin corresponding to the location of Merkel receptors to estimate the spatial activation of SA1 afferents. We then convolved this simulated spatial pattern of activation with Gabor filters to estimate the spatial variation in the SA1 response, as had been previously done with measured, rather than simulated, responses. First, we were able to replicate results from previous

neurophysiological studies demonstrating that SA1 spatial variation could account for perceived roughness. We extended these findings to predict psychophysical judgments for stimuli - varying in both inter-dot spacing and dot height - to which the SA1 responses had not been previously measured. We found that spatial variation to simulated dot patterns is highly predictive of roughness judgments for these stimuli as well. Thus, spatial variation in SA1 afferent responses can account for the perceived roughness of embossed dot patterns across all conditions for which roughness judgments have been obtained.

Disclosures: J. Goodman: None. **J. Lieber:** None. **S. Bensmaia:** None. **H. Saal:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Program#/Poster#: 441.17/JJ18

Topic: D.09. Tactile/Somatosensory

Title: Preliminary evidence for variance based information processing mechanisms in the trigeminal pathway of the albino rat

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Abstract: Here we explore information processing and coding strategies such as inter-spike variance and space-time type information coding used by vibrissae-related neurons to transmit information from the brainstem trigeminal nucleus to the ventro-postero-medial nucleus of the thalamus. We recorded the responses of pairs of thalamic-projecting neurons to trains of 1-40 Hz air-jet stimuli delivered to the vibrissae of urethane-anaesthetised rats. Recordings were analysed for recovering the spikes through non-linear curve fitting of Lorenzian spikes models. Fitted models were visually inspected for correctness. The inter-spike distances associated with each stimulation frequency were calculated and this analysis protocol was repeated for multiple recordings. The variance of inter-spike differences plotted against the different stimulation frequencies. Certain stimulation frequencies were associated with statistically higher variances that are different depending on the brain site of the recording. There was no statistically significant difference of means or medians for different stimulation frequencies. The described results suggest the relation between inter-spike variance as a mechanism for coding stimulation

associated with different frequencies. Furthermore the presence of multiple spikes that are triggered by stimulus suggest also a mechanism of space-time coding, a scheme popular in wireless communications where multiple antennas transmit redundant copies of the same information for improving the reliability of information transmission. The role of variance has been identified as a potential mechanism of information processing in previous literature as well. However, further experiments are required for confirming or rejecting the proposed information processing mechanism.

Disclosures: P. Xanthopoulos: None. **J.E. Coleman:** None. **C. Torets:** None. **F. Panetsos:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

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Lord Alliance Foundation

Title: Microsecond-scale spike timing precision in rodent trigeminal primary afferents

Authors: *M. R. BALE[1,2], A. ERSKINE[2], D. CAMPAGNER[2], R. S. PETERSEN[2]
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Abstract: Although temporal coding is implicated in all sensory modalities, spike timing precision varies greatly - from microseconds for sound localisation and electrosensation to 10s of milliseconds for vision and olfaction. In the rodent whisker system, temporal coding is evident at several stages of the sensory pathway. Whisker-object contact causes rapid fluctuations in the forces acting on the follicular mechanoreceptors and triggers reliable patterns of spikes. Since the precision of these spike time patterns is fundamentally constrained by first-order neurons, we investigated the degree and limits of temporal precision in trigeminal primary afferents in rats and mice. We found that measured spike timing precision (‘jitter’) is limited both by electrophysiological sampling rate and by the stimulation paradigm used to evoke responses. To

better estimate the physical limits of timing precision, in head-fixed mice, we measured the peak angular velocities of whiskers exploring a pole presented rapidly into their sensory field. Often, whiskers would protract (or retract) and continue moving beyond the pole causing the whisker to move with high velocity ('slip') to the other side. 17% of these slips had peak angular velocities over 10,000 degrees/s (median = 6,600 degrees/s). To match the highest velocities we observed in awake animals, we delivered an ultrafast 'ping' (>55,000 degrees/s) to a single whisker of anaesthetised mice and rats and sampled primary afferent activity at 500 kHz. Under these conditions, median spike jitter was 17.4 μs with 29% of neurons having spike jitter less than 10 μs. Our results indicate that the input stage of the trigeminal pathway has extraordinary spike timing precision that ranks among the highest in biology.

Disclosures: **M.R. Bale:** None. **A. Erskine:** None. **D. Campagner:** None. **R.S. Petersen:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Topic: D.09. Tactile/Somatosensory

Title: When gentle touch becomes pleasant

Authors: ***M. DAVIDOVIC**[1], G. STARCK[3], H. OLAUSSON[2]
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Abstract: Introduction The aim of the presented study is to describe the network of brain areas active during the perception of the pleasant touch, with the goal to lay ground for further investigations of touch in groups of patients where such perception is altered. Methods In one fMRI session consisting of 6 runs, thirty-one healthy subjects received a gentle touch stimulus in form of slow stroking with a soft brush on their right forearm. Each run consisted of ten blocks of 8-s periods of brushing followed by 12-s of rest. By using a robotic devise to deliver tactile stimuli we were able to control the pressure (0.5 N) and velocity (2 cm /s) of the stimulus, so that it remained same over the course of the sessions. Subjects were instructed to close their eyes and concentrate on brushing. After each run, subjects were asked to rate pleasantness of received stimulus on a scale between -5 (very unpleasant) and 5 (very pleasant). In the fist step of the data

analysis, a general linear model with one predictor was constructed for each run. A fixed and mixed effect models were used to generalize activations to the single subject and the group level respectively. The resulting activation maps were thresholded at FDR corrected p = 0.05. In a separate analysis pleasantness ratings were used as additional covariate. The map of voxels with high correlations to pleasantness ratings was thresholded at uncorrected p=0.001 and cluster size k=10. In the second step, spherical ROIs with radius 8 mm were constructed at peak voxels. ROI to ROI correlations were calculated using each ROI as a seed. The resulting maps were thresholded at FDR corrected p = 0.05. Results Brushing stimulus delivered by the robot was rated as pleasant (mean = 1.6, $p < 0.00001$). Brain areas with high BOLD responses include: perisylvian regions, anterior and posterior bilateral insula, anterior cingulate cortex, thalamus and ventral striatum. Significant correlation with pleasantness was found in right posterior supratemporal sulcus (pSTS) and right parietal cortex. From these activities, total of 19 ROIs were build. Correlation analysis shows that three of these ROIs show hub-like connectivity pattern: bilateral SII and right pSTS. Conclusions Our results reveal the importance of SII and pSTS in processing gentle touch stimuli.

Disclosures: **M. Davidovic:** None. **G. Starck:** None. **H. Olausson:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: Whitehall Foundation

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Title: Circuit analysis of choice-related activity in mouse somatosensory cortex

Authors: H. YANG, S. E. KWON, *D. H. O'CONNOR
Dept. of Neurosci., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: A key goal of neuroscience is to understand the mechanisms underlying perceptual decisions. A widely adopted approach is to correlate trial-by-trial spiking activity of individual neurons with behavioral choices. Choice-related activity has been observed in many cortical

areas, but its origins (and thus significance) remain unclear. We investigated choice-related activity in mouse somatosensory cortex (S1). Mice were rewarded for detecting brief deflections of the C2 whisker. We performed intracellular (whole cell) current clamp recordings in the C2 column of S1, predominantly from super- and sub-granular neurons. We compared neural responses on trials in which the mouse detected the stimulus ("hits") with trials in which the mouse failed to detect the stimulus ("misses"). A subset of S1 neurons (~33% of our sample) showed choice-related spiking. Analysis of membrane potential (Vm) revealed that most S1 neurons (~70% of our sample) exhibit prolonged choice-related subthreshold activity for hundreds of milliseconds after stimulus onset. On average, Vm was 1.5 mV more depolarized on hits compared with misses. We quantified the fraction of trials an ideal observer could correctly categorize into hits and misses based on the neural response ("choice probability"). Choice probability for Vm was on average ~0.57. Thus, there is widespread subthreshold choice-related activity that is converted into choice-related spiking in a subset of S1 neurons. To investigate the origins of this subthreshold choice-related activity in S1, we recorded multiunit activity in VPM thalamus, the primary thalamic input to S1. VPM spiking showed a transient difference between hits and misses, limited to a ~10 ms window at the peak of the stimulus-evoked response. Interestingly, VPM activity could not account for the prolonged difference in Vm observed in S1 neurons. This suggests that choice-related activity in S1 reflects recurrent activity within S1 or long-range feedback/modulatory inputs, rather than simple feedforward relay of choice-related input from thalamus. How is widespread, subthreshold choice-related activity in S1 converted to spiking in a subset of neurons? Surprisingly, spiking choice probability for a neuron was not predicted by its subthreshold Vm choice probability. Moreover, spiking choice probability was not predicted by a neuron's spike threshold, its resting potential or the difference between resting potential and spike threshold. We identified neuron-specific properties that determine choice-related spiking. Our results are explained by a simple framework that mechanistically links stimulus- and choice-related spiking.

Disclosures: **H. Yang:** None. **D.H. O'Connor:** None. **S.E. Kwon:** None.

Poster

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Catedra Marcos Moshinsky (EM)

Title: Dorsal horn neurons involved in the generation of spontaneous cord dorsum potentials exhibit phasic responses to tactile stimuli

Authors: *R. TECUANHUEY[1], D. VAZQUEZ[2], J. A. TAPIA[3], A. TREJO[4], N. HUIDOBRO[4], T. V. BALTINA[5], E. MANJARREZ[2]
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Abstract: The spinal cord exhibits background neuronal activity with transient peaks named negative spontaneous cord dorsum potentials (nSCDPs). The neurons firing in synchrony with these potentials are located in the deep spinal dorsal horn, Rexed's laminae III-VI. The aim of the present study was to characterize the frequency response of these nSCDPs-neurons to tactile stimulation on the hindlimbs skin. Experiments were performed in adult cats anesthetized with pentobarbitone. The lumbo-sacral spinal segments were exposed and the dura mater was removed. After the surgical procedures, the animals were restrained in a stereotaxic apparatus using spinal and pelvic clamps. We applied mechanical stimuli to the hindlimb skin via a closed-loop mechanical stimulator-transducer Chubbuck. The stimuli consisted of force pulses of 1 sec duration, which were adjusted to 1.5 times threshold required to evoke a visible spike response. A surface multielectrode of 30 channels was employed to identify the regions of maximal activity to tactile stimulation. Extracellular unitary recordings of dorsal horn neurons within such regions were obtained by means of glass micropipettes filled with NaCl 1.2 M (7.0 -15.0 MOhms). In other series of experiments a Minimatrix Thomas Recording multielectrode of 5 channels was employed to obtain simultaneous unitary recordings of dorsal horn neurons. We found that the nSCDPs-neurons exhibited a conspicuous phasic firing response that adapts rapidly to the tactile stimulation. Furthermore, we developed a mathematical model that accurately simulates the phasic patterns, as well as the nSCDPs. We suggest that the phasic-response property of synchronized nSCDPs-neurons contributes to the negative peak-like shape of the nSCDPs and to the coding of brief tactile stimuli.

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Poster

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Topic: D.09. Tactile/Somatosensory

Support: CIHR

GRSNC (FQRS)

NSERC

Title: Effects of transcranial direct current stimulation, tDCS, of primary somatosensory cortex, S1, on detection of vibrotactile stimuli in humans

Authors: *S. LABBÉ, E.-M. MEFTAH, C. E. CHAPMAN
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Abstract: tDCS is a non-invasive technique whereby weak, direct current stimulation is applied to different regions of cortex. This is reported to enhance, anodal (a), or decrease, cathodal (c), cortical excitability. Few studies have tested the effects of S1 tDCS on tactile perception, and the results to date are mixed. We tested the effects of tDCS (a-, c- and sham) applied to the right S1 hand representation, 2cm posterior to C4, on tactile detection of vibration (0.5 s duration, 20Hz, amplitudes of 2, 6 and 10μm) applied to the distal pad of the left middle finger. We predicted that a-tDCS would improve tactile detection (decreased threshold) while c-tDCS would have the opposite effect. Tactile detection was measured before, during and after 20 min of tDCS (1mA) in 12 subjects (8 women, 4 men; aged 18 - 25yr). A bias-free signal detection theory approach was used: half of the trials contained a stimulus; 50% had no stimulus. Subjects indicated whether a stimulus was present or not and rated their degree of confidence in this using a 5-point scale. These data were used to generate ROC (receiver operating characteristic) curves. The area under the ROC curves was calculated for each vibrotactile intensity. From this we interpolated detection threshold (0.75). Mean baseline detection threshold was 4.3 μm (range 1 to 7μm) in 12 subjects. With a-tDCS, six subjects showed the predicted decrease in threshold either during or immediately after tDCS (to 38% of control). For this group, there was a significant difference across the 3 conditions (ANOVA, $p=0.018$). Post-hoc contrasts showed that there was a significant difference between anodal t-DCS and the other 2 conditions ($p < 0.0005$).The same subjects showed no change in threshold for c-tDCS and sham ($p=0.198$). Six other subjects showed no effect in any condition. The results are consistent with a-tDCS enhancing S1 cortical excitability, and so improving tactile detection threshold. In contrast, c-tDCS had no effect on tactile detection suggesting that there was no change in cortical excitability with this polarity of stimulation.

Disclosures: **S. Labbé:** None. **E. Meftah:** None. **C.E. Chapman:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

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Title: Stimulus preference profiles of whisker sensitive neurons in trigeminal nuclei

Authors: *S. CHAKRABARTI[1,3], A. MAIA-CHAGAS[1,3], C. SCHWARZ[2,3]
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Abstract: The rat brainstem trigeminal nuclei principalis (Pr5) and interpolaris (Sp5i) contain whisker sensitive neurons that represent the first synaptic station on the ascending somatosensory pathway originating from the whisker primary afferents and terminating in the primary somatosensory cortex. From a previous study, we know that primary afferents (TG) convey enormous amounts of information about broad-spectrum dynamic whisker deflections (Chagas et al., 2013). In the present study we were interested to quantify, how much of this information is preserved in Pr5 and Sp5i. We recorded well isolated single units from the Pr5 and Sp5i of anesthetized rats during single whisker stimulation using a white noise stimulus. We computed instantaneous information transfer from spike triggered ensembles of the kinematic parameters position, velocity and acceleration). The time interval between stimulus time series and response at which instantaneous information rate reached a maximum indicated the neuron's response latency. Further using a novel neuronal encoding model (Theis et al., 2013) we calculated the mutual information between the spike train and the different stimulus kinematic parameters to determine which parameter or parameter combination yielded the highest information transfer. Our results obtained so far showed Pr5 and Sp5i neurons convey information about the three tested kinematic parameters and show an optimal latency above 2 ms expected from the synaptic delay. Importantly information rate about the stimulus drops considerably from TG to both Pr5 and Sp5i neurons, possibly reflecting considerable neuronal computation in the brainstem network and/or integration of non-sensory signals. Grant Support DFG SCHW577/10-2 and

DFG CH 1232/1-1 References: Chagas et al., Front Neur Circuits, 2013 Theis et al., pLOS Comp Biol, 2013

Disclosures: S. Chakrabarti: None. **A. Maia-Chagas:** None. **C. Schwarz:** None.

Poster

441. Somatosensory: Stimulus Feature Neural Coding

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 441.24/JJ25

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant HD46922

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Title: Muscle spindles encode force information

Authors: *K. P. BLUM[1,2], B. LAMOTTE D'INCAMPS[3], D. ZYTNICKI[3], L. H. TING[1,2]
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Abstract: The encoding of force or acceleration in muscle spindle primary afferents has been suggested but never definitively demonstrated. There is also no consensus on roles of the initial burst and of history-dependence on the information that is encoded. Moreover, their physiological relevance is unclear. However, muscle responses to balance perturbations that arise from group I afferents resemble history-dependent initial bursts and are sensitive to acceleration (Lockhart and Ting 2007; Safavynia and Ting 2013). Here, we hypothesized that detailed dynamics of muscle spindle instantaneous firing rates (IFRs) encode information related to acceleration of the spindle-bearing muscle. We applied ramp and hold stretch perturbations to the triceps surae muscle-tendon complex of anesthetized cats while recording from group I spindle afferents in the dorsal column using sharp recording techniques. To test the dependence of the initial burst on acceleration, a variety of peak acceleration and velocity profiles were applied, with rest periods of 5-10 s between perturbations. To test history dependence, sawtooth perturbations were applied. Whole musculotendon force and its first time derivative (dF/dt) were also measured. Data from 9 Ia afferents were obtained, with 58 to 417 perturbations per afferent. In 4 afferents with prominent initial bursts, the initial burst amplitude was correlated to both peak

acceleration and peak dF/dt ($p < 0.001$ for each afferent). For all 9 afferents, we fit linear models of whole-muscle kinematics (length, velocity and acceleration) and whole-muscle kinetics (force and dF/dt) to the IFRs. We used Akaike information criteria (ΔAIC) and R^2 to compare the ability of each model to explain all of the data from each afferent using a single set of parameters. For 6 out of 9 afferents, the kinematic model was much less likely to be representative of the experimental data than the kinetic model (ΔAIC = 4.75 ± 2.21, R^2 = 0.906 ± 0.05 (kinetic) and 0.651 ± 0.24 (kinematic)). For the other 3 afferents, neither model was more likely to explain the experimental data (ΔAIC = 1.95 ± 0.057, R^2 = 0.564 ± 0.075 (kinetic) and 0.693 ± 0.040 (kinematic)). In contrast to our hypothesis, the detailed dynamics and history dependence of Ia muscle spindle IFRs reflect musculotendon kinetics rather than kinematics. Differences between the models were more pronounced during dynamic versus static responses to perturbation. Moreover, initial burst and history dependence of the IFRs resembled force nonlinearities previously observed in muscle fibers. We conclude that as a proxy for muscle acceleration, velocity, and displacement information, muscle spindles use the force.

Disclosures: K.P. Blum: None. **B. Lamotte d'Incamps:** None. **D. Zytnicki:** None. **L.H. Ting:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 442.01/JJ26

Topic: D.15. Basal Ganglia

Support: ANR Grant 10-PDOC-016-01

EU Grant PIRG-GA-2009-256488

Title: A cerebello-basal ganglia pathway involved in song learning

Authors: L. PIDOUX[1], C. LEVENES[1], *A. LEBLOIS[2]
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Abstract: Human speech is a complex sensorimotor skill and vocal learning is one of the most striking cognitive abilities of the brain. As many other complex motor skills, vocal learning involves the basal ganglia (BG)-thalamo-cortical network and the cerebello-thalamo-cortical network in humans. While the BG and cerebellar sub-cortical loops have been shown to interact

through at least two different pathways in mammals, the role of their interaction during sensorimotor learning, and in particular during vocal learning, remains undetermined. Songbirds are one of the few accessible animal models for vocal learning, as they have a specialized portion of their BG-thalamo-cortical circuitry dedicated to song learning. Additionally, a cerebellar projection to the thalamic region adjacent to the song-related thalamic nucleus receiving BG input suggests that BG and the cerebellum may interact during song learning. However, very little is known about song-related circuits in the cerebellum, or about a putative cerebellar function in song learning. In order to determine to what extent the cerebellum is involved in song learning and dissect the cerebellar circuits interacting with thalamic and cortical song-related nuclei, we performed two sets of experiments. On one hand, we investigated the physiological mechanisms underlying the transfer of cerebellar signals from deep cerebellar nuclei to the song-related BG nucleus Area X in adult zebra finches. To this end, we recorded the evoked activity in Area X output neurons (pallidal cells) following electrical stimulation in the deep cerebellar nuclei. Stimulation of the deep cerebellar nuclei evoked fast excitatory responses in pallidal neurons located in Area X. We then combined the recordings and stimulation with pharmacological blockade of synaptic transmission in the putative thalamic relay nucleus (DTZ) and in Area X. Our results suggest that cerebellar signals are transmitted via a glutamatergic pathway through the thalamus. On the other hand, we performed lesions in the deep cerebellar nuclei of adults and juveniles zebra finches to probe the putative function of the cerebellum during song production and learning. While lesion induced minor or no change in adult zebra finch song, it impaired song learning in juvenile zebra finches (lesions applied around 60 days old). Our results suggest that BG and cerebellar circuits cooperate for efficient song learning in songbird and open a new pathway to address cerebellar function in this promising animal model.

Disclosures: L. Pidoux: None. **C. Levenes:** None. **A. Leblois:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 442.02/JJ27

Topic: D.15. Basal Ganglia

Support: The Maxwell Fund

Title: Connections of dorsocentral striatum with substantia nigra: Circuitry for directed attention

Authors: S. A. SZYMANSKI, *R. L. REEP
Univ. Florida, GAINESVILLE, FL

Abstract: Dorsocentral striatum (DCS) is an associative region of the striatum that is involved in directed attention and its dysfunctional counterpart, contralateral neglect. DCS receives major cortical input from medial agranular (AGm) and posterior parietal cortex. Area AGm influences orienting movements of the head and neck through projections to superior colliculus and the cervical spinal cord. DCS is known to project to substantia nigra pars reticulata (SNr) and globus pallidus (GP), which play a central role in orienting head movements that are made toward external stimuli, through their connections with the superior colliculus and thalamic nuclei. Behavioral and pharmacological studies have shown that DCS is essential for recovery from neglect, whether spontaneous or induced. Because of the essential role of DCS in directed attention, we sought to better define the topography of projections from DCS to GP and SNr. Small deposits of 10% biotinylated dextran amine were made in DCS or surrounding regions of the dorsal striatum in 28 male Long Evans hooded rats. Following survival times of 7-10 days, animals were sacrificed and the brains were processed for the presence of BDA-labeled axons. The projection to the dorsal portion of globus pallidus (GPd) exhibited a topographic organization that reflected the dorsoventral and mediolateral location of the injection site in the dorsal striatum, with substantial overlap in the central region of GPd. No rostrocaudal topography was discerned. Within substantia nigra pars reticulata (SNr), there appeared to be less topography. Injections in DCS produced labeling focused in ventromedial SNr. Injections that were located medial or lateral to DCS resulted in labeling in SNr that was correspondingly shifted to some extent, but overlap within ventromedial SNr dominated these effects. Ventromedial SNr is known to project to medial and caudal regions of the superior colliculus, corresponding to the upper visual field and temporal periphery, which plays a role in saccadic eye and head movements. The superior colliculus, and thus ventromedial SNr, have an important role in sensory processing, detection of novel events, and responses to these events. This circuit, through its connections with the superior colliculus, is a significant aspect of the way the forebrain influences head and eye movements.

Disclosures: S.A. Szymanski: None. **R.L. Reep:** None.

Poster

442. Systems Physiology and Circuits

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Title: Laminar and regional distribution of cortical neurons innervating striatonigral neurons in rats as determined using transneuronal retrograde transport of rabies virus

Authors: *Y. DENG[1], J. L. LANCIEGO[2], L. KERKERIAN-LE GOFF[3], P. COULON[4], P. SALIN[3], P. KACHIDIAN[3], A. J. REINER[1]
[1]Univ. of Tennessee HSC, MEMPHIS, TN; [2]Neurosciences Division, Ctr. for Applied Med. Res. (CIMA and CIBERNED), Univ. of Navarra Med. Col., Pamplona, Spain; [3]Aix-Marseille Université, CNRS, IBDM UMR7288, 13009, Marseille, France; [4]Aix Marseille Université, CNRS, INT UMR 7289, Marseille, France

Abstract: Cortical input to striatum arises from two neuron types: 1) Intratelencephalically projecting (IT-type) layer 5a neurons that are involved in pre-movement planning; 2) Pyramidal tract (PT-type) layer 5b neurons that transmit motor commands to hindbrain and spinal cord. In prior studies in rats, we have shown that the dendritic spines of direct pathway neurons preferentially receive input from IT-type neurons (Reiner et al., Frontiers 2010). In the present study, we have injected Challenge Virus Standard (CVS-11) rabies virus (RV) into rat substantia nigra pars reticulata to take advantage of the trans-synaptic spread of RV to identify the cortical neurons projecting to direct pathway striatonigral projection neurons (dSPNs). The post-injection survival time (40-42 h) was chosen to limit labeling to cortical neurons projecting directly to striatonigral neurons. From analysis of cortex at levels through the basal ganglia in 5 cases (10-15 sections per case), we found that corticostriatal neurons projecting to dSPNs of dorsal striatum mainly reside in M1/M2 (65%) and S1/S2 (10%), with the contralateral neuron abundance being 10% of the ipsilateral abundance. Labeled neurons in upper layer 5 of M1/M2 were in a 3:1 ratio with lower layer 5 labeled neurons in contralateral cortex, and in a 5:3 ratio for contralateral S1/S2. Since contralateral RV-labeled cortical neurons should all be IT-type (Reiner et al., Frontiers 2010), and PT-type neurons do not project contralaterally to striatum, this result suggests that the contralateral IT-type input arises mainly but not exclusively from upper layer 5. The upper to lower layer 5 ratio for ipsilateral M1/M2 is the same as for

contralateral M1/M2 (3:1), but the upper layer 5 to lower layer 5 ratio for ipsilateral S1/S2 is 1:1. These results indicate that dSPNs receive mainly IT-type input from M1/M2 ipsilaterally and contralaterally, but the greater proportion of lower layer 5 neurons ipsilaterally than contralaterally suggests ipsilateral S1/S2 input to dSPNs includes both IT-type and PT-type. Thus, S1/S2 at least is seemingly a major source of PT-type input to dSPNs. The overall results suggest that dSPNs do preferentially receive input from IT-type cortical neurons, but by their nature the data cannot clarify the relative abundance of axospinous versus axodendritic versus axosomatic IT-type synaptic terminals on dSPNs from any given regional or laminar source. Nonetheless, unlike in Wall et al. (Neuron 2013) in mice, we find that dSPNs do receive a major input from motor cortex in rat, as consistent with their role in motor initiation.

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Poster

442. Systems Physiology and Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 442.04/JJ29

Topic: D.15. Basal Ganglia

Support: NIH Grant NS23805

Title: Specificity of laterobasal amygdaloid projections to striatum and the extended amgydala

Authors: *R. A. REICHARD, K. P. PARSLEY, D. S. ZAHM
Pharmacol. and Physiological Sci., St. Louis Univ. Med. Sch., Saint Louis, MO

Abstract: The basal and accessory basal (a.k.a., basolateral and basomedial; laterobasal) nuclei of the amygdala (LB) project very densely and in a topographically ordered fashion to both the central division of the extended amygdala (EAc) and striatum, as well as less densely to the lateral septum. We previously reported that significant numbers of orbitofrontal and insular cortical neurons project by way of axon collaterals to sites relatively distant from each other but within the bounds of either the EAc or striatum, whereas fewer neurons have axon collaterals projecting to both the EAc and striatum (J Neurosci 25:11757, 2005). In constrast, we observed later that the subiculum of the hippocampus, which projects very densely to the lateral septum and accumbens shell, a subterritory of the striatum, exhibits a substantial number of neurons that projects to both (Soc Neurosci Abst 491.22, 2010). This different pattern of collateralization may

reflect the substantial number of axonal interconnections between the lateral septum and accumbens shell and that the accumbens shell harbors significant numbers of neurons with lateral septum-like downstream projections, suggesting an intermixing of the two structures (J Comp Neurol 219:511, 2014). The present study was done to assess whether outputs to the EAc, striatum and lateral septum from the LB emulate the pattern of the orbitofrontal/insular cortex or hippocampus. The study involved injecting distinct retrograde tracers into pairs of sites and evaluating relative numbers of double labeled neurons in the LB with the aid of the Neurolucida hardware-software platform. Material was used from rats injected in our earlier studies (J Neurosci 2005 and SfN 2010, as above) in which retrograde tracers FluoroGold and cholera toxin beta subunit were used to generate brown and black immunoperoxase products. Whereas the analysis of that material is subject to confirmation with fluorescence microscopy in additional cases to be injected with cholera toxin conjugated to distinct fluorescent molecules, the preliminary data indicate that injection pairs with both injection sites occupying either EAc or striatum are associated with greater numbers of double-labeled neurons than injection pairs with equivalently distant injection sites of which each is in a different macrosystem. These data, consistent with the 2005 J Neurosci paper, suggest that EAc and striatum subserve separate subpopulations of LB output neurons, rather than differentially processing outputs from the same population of LB neurons. Interestingly at present, all LB projections to the lateral septum appear to be collaterals of accumbens shell-projecting LB neurons.

Disclosures: **R.A. Reichard:** None. **K.P. Parsley:** None. **D.S. Zahm:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

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Program#/Poster#: 442.05/JJ30

Topic: D.15. Basal Ganglia

Support: NIH Grant NS23805

Title: A novel afferent of the ventral tegmental area and substantia nigra compacta from the deep frontal lobe

Authors: ***D. ROBY**, L. YETNIKOFF, K. P. PARSLEY, D. S. ZAHM
Pharmacol. and Physiological Sci., St. Louis Univ., St Louis, MO

Abstract: In several reports on the afferents of the ventral tegmental area (VTA) as revealed with retrograde tracers (e.g., J Comp Neurol 490:270-294, 2005; Eur J Neurosci 24:116-134, 2006; J Neurosci 27:5730-5743, 2007; J Comp Neurol 522:1031-1047, 2014), we have described "densely packed···neurons···in an area that Paxinos and Watson (1998) identified as dorsal peduncular cortex" and "moderate numbers···in the rostral claustrum/endopiriform nucleus complex···that···are arranged as a thin band of labeled cells near the corpus callosum (J Comp Neurol 490:270-294, 2005; see Fig. 11 therein). Despite the apparent cohesiveness of this strikingly dense collection of VTA-projecting neurons, it has remained relatively neglected. It's rostral extremity is a small, band-like aggregation of retrogradely labeled neurons that caps the anterior commissure in the olfactory peduncle just in front of the rostralmost tip of the accumbens, which, in company with the olfactory ventricle and its subependymal sheath, insinuates between the cluster and anterior commissure. As the Acb increases in size in successively caudal sections, the aggregation of VTA-labeled neruons is very dense and drapes over the accumbal dorsomedial and dorsolateral aspects into continuity medially with a deepest, thin lamina of the medial prefrontal cortex (mPfC) and laterally with deep parts of the endopiriform/claustrum continuum, essentially ensheathing the medial and lateral aspects of the callosal forceps minor. This cluster of VTA-labeled neurons is conspicuous because the intensity of the labeling of its individual neurons substantially exceeds that of VTA-labeled neurons in adjacent parts of the mPfC and Acb, particularly in younger rats (J Comp Neurol 522:1031-1047, 2014). Iontophoretic injections of the bi-directional axonal tracer cholera toxin beta subunit into the cell dense center of the VTA-labeled cluster where it overlies the rostral tip of the Acb revealed very strong reciprocal connections with the olfactory bulb, entire extents of the piriform and agranular, granular and dysgranular insular cortex, thalamic reuniens and submedius nuclei, and the VTA. Retrograde labeling was observed in numerous other structures, such as the lateral preoptic area and laterobasal amygdala. The projection to the ventral mesencephalon, which was confirmed with PHA-L injections, encompasses the VTA and substantia nigra compacta (SNc) and is very dense, but rather inconspicuous by virtue of comprising a plexus exclusively of exceedingly fine gauge axons. These data suggest a projection to the VTA and SNc of previously unforeseen significance, likely conveying multimodal sensory information.

Disclosures: **D. Roby:** None. **L. Yetnikoff:** None. **K.P. Parsley:** None. **D.S. Zahm:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

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Topic: D.15. Basal Ganglia

Support: NIH Grant DA03906

NIH Grant DA12513

Title: The Indirect Pathway is not what you think: D1 medium spiny neurons of the nucleus accumbens project to the ventral pallidum

Authors: *Y. M. KUPCHIK, R. M. BROWN, D. SCHWARTZ, P. W. KALIVAS
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: The nucleus accumbens (NAc) serves as the input structure of the basal ganglia and is central to addictive behavior. Information entering the NAc is processed and then sent to output structures of the basal ganglia (such as the substantia nigra (SN) and the ventral tegmental area (VTA)) in two parallel pathways: a direct pathway that consists of medium spiny neurons (MSNs) directly innervating the output structures and an indirect pathway, in which the striatal axons first innervate the ventral pallidum (VP), which then sends axons to the output structures. The direct pathway is classically considered to consist solely of MSNs expressing the D1-dopamine receptor (D1-MSNs), while the indirect pathway consists solely of MSNs expressing the D2-dopamine receptor (D2-MSNs). Here, we used whole-cell patch clamp electrophysiology to test whether the widely accepted division of D1- and D2-MSNs into direct and indirect pathways is accurate. By injecting Cre-dependent channelrhodopsin (ChR2) into the nucleus accumbens core (NAcore) of D1- or D2-Cre mice and recording from VP neurons we found that ~50% of VP cells were innervated by D1-MSNs while almost all VP cells were innervated by D2-MSNs. In contrast, cells in the SN received only D1-MSN input from accumbens, while the globus pallidus received less than 10% D1-MSN input from the dorsal striatum. To examine the possibility that accumbens D1-MSNs form an indirect pathway through the VP we retrogradely labeled VP neurons projecting to the VTA/SN and optogenetically activated D1-MSN terminals in the VP while recording from retrogradely labeled cells. We found that a substantial proportion of VTA/SN-projecting VP neurons received input from D1-MSNs. These data prove that D1-MSNs comprise a substantial part of the indirect pathway. Thus, our view of the direct and indirect pathways of the ventral striatum needs revision, with emphasis on the VP as an integration point of the majority of the NAc output. Further examination of the role of the different accumbal inputs to the VP in addiction will determine the behavioral importance of the newly-discovered D1-MSN input to the VP.

Disclosures: Y.M. Kupchik: None. **R.M. Brown:** None. **D. Schwartz:** None. **P.W. Kalivas:** None.

Poster

442. Systems Physiology and Circuits

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Program#/Poster#: 442.07/JJ32

Topic: D.15. Basal Ganglia

Support: CIHR MOP-130393

Title: Disinhibition of prefrontal cortex differentially gates hippocampal and amygdala inputs to the nucleus accumbens

Authors: *M. TSE[1], S. B. FLORESCO[2]
[2]Psychology, [1]Univ. British Columbia, Vancouver, BC, Canada

Abstract: Pathophysiological alterations in prefrontal cortex (PFC) GABA transmission have been proposed to underlie various psychiatric disorders. Previous studies in our laboratory have revealed that pharmacological reduction of PFC GABA activity can produce a variety of cognitive, affective and dopaminergic abnormalities that resembles schizophrenia, including impaired spatial memory (mediated by the hippocampus) and aberrant attributions of salience to fear-related stimuli (mediated by the amygdala). Inputs from the PFC, hippocampus and amygdala converge within the nucleus accumbens (NAc), yet the manner in which disinhibitory increases in PFC outflow may affect integration of cognitive and emotional information arising from these temporal lobe inputs remains to be elucidated. In the present study, we recorded from NAc neurons that that received inputs from either the hippocampus or the basolateral amygdala (BLA) in urethane-anesthetized rats. Under basal conditions, stimulation of fimbria/fornix (conveying hippocampal output) and the BLA reliably evoked spike firing in separate populations of NAc neurons. Disinhibition of the PFC via local infusion of the GABA-A antagonist bicuculline (25-50ng) reliably attenuated hippocampal evoked firing. In contrast, reducing PFC GABA transmission did not reduce firing evoked by BLA stimulation in a separate population of cells, and actually lead to an enhancement in evoked firing in some neurons. This suggests that reduced PFC GABA activity may differentially gate mnemonic versus emotional signals originating from the temporal lobes and converging in the NAc. Furthermore, they suggest perturbations in PFC GABA transmission that may occur in schizophrenia may lead to altered gating of these inputs to the NAc. This in turn may contribute to impairments in hippocampal-mediated cognitive function and aberrant affective salience attribution and increased anxiety observed in the disorder.

Disclosures: M. Tse: None. **S.B. Floresco:** None.

Poster

442. Systems Physiology and Circuits

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Program#/Poster#: 442.08/JJ33

Topic: D.15. Basal Ganglia

Support: CIHR MOP-133579

Title: Lateral habenula stimulation overrides evoked phasic bursting of dopamine neurons

Authors: *C. M. STOPPER[1], M. T. L. TSE[2], S. B. FLORESCO[2]
[2]Psychology, [1]Univ. British Columbia, Vancouver, BC, Canada

Abstract: The lateral habenula (LHb) serves a critical role in reward prediction error. Neurons in this region display an inverted signal to that of midbrain dopamine (DA) neurons, with increased firing observed after reward omission and decreased firing after reward or reward-predictive cues. Stimulation of the LHb briefly inhibits ventral tegmental area (VTA) DA neuron firing , indicating that this nucleus may drive phasic inhibition of DA cells, which may be important for phasic dips of DA activity following omission of expected reward, a process known as negative reward prediction error (nRPE). While it is known that the LHb encodes an inverted nRPE signal and stimulation of this region can inhibit spontaneous DA activity, the power of this signal to override phasic bursting of DA neurons that might occur during unexpected reward has not been explored. Recent behavioral data from our laboratory demonstrate that stimulation of the LHb during reward delivery on a probabilistic choice task redirects the direction choice as might be expected from overriding phasic DA bursts. The current experiments aimed to verify the importance of DA for this effect and to determine the power of LHb stimulation as a tool to occlude phasic DA bursting. Male Long-Evans rats, anesthetized with chloral hydrate were implanted with microelectrodes in the VTA for extracellular recording of DA neurons and stimulating electrodes in the LHb as well as the medial prefrontal cortex (mPFC) and pedunculopontine tegmental nucleus (PPTg), two regions that can promoting burst-firing by DA neurons. As expected, 4-pulse or 20-pulse train stimulation of the LHb (700 μA, 100 Hz) briefly inhibited spontaneous DA neuron firing. Single-pulse stimulation of either the mPFC or PPTg evoked excitation in DA neurons. In these same cells, stimulation of the LHb, terminating 10 ms prior to mPFC or PPTg stimulation, inhibited or greatly attenuated evoked DA neuron firing. These findings demonstrate the power that brief, but precisely-timed, LHb stimulation has on phasic DA activity driven by excitatory inputs. As this signal is critical for reward prediction error, LHb stimulation shows promise for rectifying disadvantageous decision biases underlying various pathologies characterized by DA dysfunction.

Disclosures: **C.M. Stopper:** None. **M.T.L. Tse:** None. **S.B. Floresco:** None.

Poster

442. Systems Physiology and Circuits

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Program#/Poster#: 442.09/JJ34

Topic: D.15. Basal Ganglia

Support: Israel Science Foundation (ISF) Grant 743/13

Tourette Syndrome Association (TSA) Grant

Title: Cortical involvement in tic generation: A behavioral and neurophysiological study in rats

Authors: M. ISRAELASHVILI, *I. BAR-GAD
Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Motor tics are repetitive, involuntary brief muscle contractions which interfere with ongoing behavior and appear as a symptom in several disorders, such as Tourette syndrome. Tics have been associated with abnormalities in the cortico-basal ganglia system, and specifically with abnormal inhibition within the striatum. We have recently demonstrated that motor tics can be induced in rodents by local micro-injection of bicuculline (GABA-A antagonist) into the dorsolateral striatum leading to focal disinhibition. The evoked motor tics are associated with phasic changes of neuronal activity throughout the cortico-basal ganglia loop which are accompanied by slower local field potential (LFP) spikes reflecting a wider population activity. In the current study we utilized this model to study the behavioral manifestation and the neuronal correlates of motor tic generation in freely moving rats following the manipulation of cortical input to the basal ganglia. Cortical excitation changes the expression of the bicuculline generated tics and leads to tic related activity changes in individual neurons and in striatal LFP. Tic related activity was found in the medium spiny projection neurons, as well as in different striatal interneuron populations including the fast spiking interneurons and the tonically active neurons. Thus, the current findings provide new insights into the role of the cortico-striatal pathway in tic generation and the underlying mechanisms of tic formation.

Disclosures: **M. Israelashvili:** None. **I. Bar-Gad:** None.

Poster

442. Systems Physiology and Circuits

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Topic: D.15. Basal Ganglia

Support: Intramural Research Program of the National Institutes of Health and National Institute of Mental Health

Title: Decorrelated striatal resting state maintained by feedback inhibition

Authors: A. KLAUS, *D. PLENZ
Sect Critical Brain Dynamics, Natl. Inst. of Mental Health, NIH, BETHESDA, MD

Abstract: One of the main forebrain structures that controls motor actions is the cortico-basal ganglia-thalamic loop. The striatum is the first subcortical stage in this loop. It consists mainly of inhibitory neurons and processes cortical inputs via two major circuits: (i) a feedback circuit of spiny projection neurons (SPNs) inhibiting nearby SPNs, and (ii) a feedforward circuit of fast-spiking interneurons inhibiting SPNs. Here, we investigate how these circuits process resting state activity of the cortex in the form of neuronal avalanches. In unrestrained rats not engaged in any behavioral task, we found that reducing local striatal inhibition induced tics in line with previous reports. Specifically, local striatal injection of picrotoxin (PTX, 1 mM, 0.9-1.5 µl; n=7), which reduces both feedback and feedforward inhibition, led to stereotypical tics at ~0.5 Hz in the contralateral front-paw that often involved head and neck. In contrast, local injection of IEM-1460 (IEM, 5 mM; n=6), which decreases feedforward inhibition by blocking AMPA-mediated input to striatal interneurons, induced tics in the contralateral front-paw at ~4 Hz that were less stereotypical. Simultaneous chronic recordings from microelectrode arrays demonstrated IEM- and PTX-tics to correlate with multi-unit firing and the local field potential (LFP) in both cortex and striatum. Tic epochs revealed spatiotemporal LFP patterns in cortex that deviated from neuronal avalanches, were more variable under IEM-tics, and less variable during PTX-tics. Thus, imbalanced striatal inhibition recruits cortico-basal ganglia loops resulting in behavioral stereotypies. We further identified the nature of imbalance originating from both striatal circuits using organotypic cortex-striatum-midbrain cultures. In this *in vitro* system, contributions from cortico-basal ganglia-thalamic loops are removed and cortical neuronal avalanche input transitions striatal neurons spontaneously through up- and down states. Calcium imaging of up to 70 striatal neurons (300x400 µm area; OGB BAPTA-1) revealed a decorrelated resting state of the striatum characterized by low average pairwise correlations in striatal neuron firing. Local, striatal application of PTX (n=8) increased and synchronized neuronal firing. In contrast, IEM

(n=11), which was confirmed to reduce spontaneous firing in cultured striatal interneurons, did not abolish the decorrelated striatal resting state. However, IEM changed individual pairwise correlations among striatal neurons. Our results identified a decorrelated resting state of the striatum that is maintained by feedback inhibition between striatal SPNs.

Disclosures: **A. Klaus:** None. **D. Plenz:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 442.11/JJ36

Topic: D.15. Basal Ganglia

Title: Optogenetic assessment of synaptic mechanisms influencing ventral striatal information integration

Authors: ***J. M. BROOKS**, P. O'DONNELL
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Abstract: The ventral striatum (VS) plays an important role in the facilitation of goal-directed behavior by effectively incorporating diverse and dynamic afferent information. Striatal medium spiny neurons (MSN) serve as a site of convergence for multiple brain regions involved in goal-directed behavior, including the prefrontal cortex (PFC) and hippocampus (HP). Several recent studies suggest these distinct excitatory inputs may differentially influence striatal circuitry in an activity dependent manner. For example, electrophysiological recordings from anesthetized rats revealed robust PFC stimulation leads to a reduction in ongoing HP-evoked MSN responses, in part, through the recruitment of local inhibitory mechanisms. Based on these data we speculate that burst-like cortical activity is capable of attenuating weaker, competing excitatory input locally within the striatum. However, the neural mechanisms guiding this complex interaction remain unclear. The current experiments were designed to explore possible synaptic mechanisms involved in PFC-evoked heterosynaptic suppression of MSN responses to competing excitatory synaptic inputs. Whole-cell current-clamp recordings were performed from rats receiving bilateral hippocampal injections of a viral vector (AAV) expressing channelrhodopsin 2 (ChR2) under the CamKinase II promoter. Input interactions between electrical stimulation of PFC fiber tracts and optical stimulation of HP inputs expressing ChR2 were tested in VS MSNs. Exposure to a light pulse (1 ms; 475 nm) reliably evoked EPSPs in VS MSNs which were reduced at a short (50 ms), but not long (500 ms), latency following burst-like electrical stimulation of

corticostriatal fiber tracts (5 pulses, 20 Hz, 0.1-0.5 mA). Furthermore, the magnitude of PFC-evoked suppression was reduced, but not completely reversed, by bath application of the $GABA_A$ antagonist, pictrotoxin (100 uM), similar to previous *in vivo* results. As the reduction is not complete, we assessed the role of endocannabinoid retrograde signaling as an alternative mechanism of action. In the VS, activation of CB1 receptors reduces presynaptic glutamate and GABA release. Bath application of the CB1 receptor antagonist AM251 (2 uM) enhanced cortical suppression of optically evoked HP responses suggesting the locus of action for AM251 is on inhibitory interneurons. Taken together, these findings further substantiate the assertion that shifts in VS neuronal activity involve local inhibitory mechanisms and provide evidence for a modulatory role of endocannabinoids in this interaction.

Disclosures: J.M. Brooks: None. **P. O'Donnell:** None.

Poster

442. Systems Physiology and Circuits

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Topic: D.15. Basal Ganglia

Support: NIH NS83815

Title: Functional dissection of cortical inputs to striatal interneurons with modified rabies virus

Authors: *J. R. KLUG[1], N. M. TAYLOR[1], F. OSAKADA[2], E. M. CALLAWAY[2], X. JIN[1]
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Abstract: The integration of information in neural circuits requires the precise coordination of activity of projection neurons and interneurons. In the striatum, the main projection neurons - medium spiny neurons (MSNs), receive inputs from the cortex and thalamus as well as from striatal interneurons. Striatal interneurons allow local modulation of MSN neuronal firing and thus contribute to action sequence learning and selection, while alterations in their function are associated with many neurological disorders including Parkinson's disease and dystonia. Fast spiking parvalbumin-containing GABAergic interneurons influence MSN activity via feed forward inhibition, strongly shaping the output of MSNs. Alternatively, large aspiny cholinergic interneurons are thought to modulate MSN synaptic transmission and plasticity via the release of acetylcholine. While striatal interneurons are important players in striatal physiology and synaptic plasticity, little is known about their input connectivity/specificity and their

contributions to behavior. In order to address these fundamental questions, we used AAV-Cre-dependent helper viruses along with an EnvA-pseudotyped, glycoprotein (G) - deleted recombinant rabies virus (SAD delta G Rabies) to selectively visualize and manipulate monosynaptic inputs to fast spiking or cholinergic striatal interneurons. Helper viruses were injected into the dorsal striatum of mice expressing CRE recombinase under the control of parvalbumin (PV-Cre) or choline acetyltransferase promoters (ChAT-Cre). A following injection of modified rabies virus allowed retrograde monosynaptic tracing and expression of Channelrhodopsin-2 (ChR2-mCherry) in presynaptic neurons. We found wide distributions of labeled presynaptic neurons in the frontal and motor cortex, intralaminar nuclei of thalamus and external globus pallidus. Functional expression of ChR2 in presynaptic neurons was verified with electrophysiological recording in brain slices, and functional specificity of their inputs was assessed in relation to the striatal direct versus indirect pathway. Optogenetic stimulation in freely moving mice was utilized to examine the contribution of interneurons, as well as their presynaptic inputs from different cortical regions, to locomotor behavior and operant learning. Together with our previous experiments functionally dissecting cortical inputs to striatal D1- versus D2-MSNs (Klug et al. 2013 SFN abstract 270.07/XX1), these results provide a comprehensive functional map of corticostriatal subcircuits, and start to reveal the computation logic of striatum and its inputs in controlling action.

Disclosures: **J.R. Klug:** None. **N.M. Taylor:** None. **F. Osakada:** None. **E.M. Callaway:** None. **X. Jin:** None.

Poster

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Topic: D.15. Basal Ganglia

Support: NIH NS83815

Title: Functional specificity and heterogeneity of basal ganglia pathways during action selection

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Abstract: Action learning is fundamental for organisms to acquire new skills for survival and welfare. Skilled behaviors are built up on spatiotemporally precisely controlled actions, and the

basal ganglia circuit has been implicated to be critical in action learning and selection. The major input nucleus of basal ganglia, striatum, comprises two subtypes of GABAergic projection neurons known as medium spiny neurons (MSNs), expressing dopamine D1 receptors (direct pathway) and dopamine D2 receptors (indirect pathway) respectively. The canonical model of basal ganglia function suggested that these two pathways work antagonistically to control actions, with direct pathway directing "Go" and indirect pathways mediating "No-Go". Another input nucleus of basal ganglia, subthalamic nucleus (STN), was thought to be involved in "stopping" or "cancelling" action, racing with direct pathway with opponent signals for inhibiting movements promptly and globally. Recent findings have demonstrated that direct and indirect pathways were co-activated during action sequence initiation and termination while functionally segregated during sequence execution, indicating dynamic interactions cross pathways might underlie complex behavior. However, how do direct, indirect and hyperdirect the three pathways interact and work together during action learning remains largely unknown, and their respective contribution (e.g. indirect vs. hyperdirect pathway) to action selection is unclear. To dissect the functional role of these three pathways, we trained mice to perform a new version of "Go/No-Go" behavioral task, in which the animal was cued to go pressing different levers or stop acting contingent on corresponding sensory stimuli. Channelrhodopsin (ChR2) was expressed in D1-cre, A2a-cre and CaMKII-cre mice to selectively target striatal D1-MSNs, D2-MSNs and STN neurons respectively. Multiple-electrode *in vivo* recording and light-assisted cell identification was utilized to record neuronal activity and identify the cell type in each pathway during the action selection. The study discovered functional specificity as well as heterogeneity of different basal ganglia pathways during action selection, revealing a more complex picture of basal ganglia circuits and function than previously appreciated.

Disclosures: H. Li: None. **X. Jin:** None.

Poster

442. Systems Physiology and Circuits

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Program#/Poster#: 442.14/KK3

Topic: D.15. Basal Ganglia

Support: NIH NS83815

Title: Subthalamic regulation of striatal dopamine and behavior

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Abstract: The basal ganglia, a collection of subcortical nuclei involved in action selection, motivation, and motor control, contain three primary and distinct subcircuits called the direct, indirect, and hyperdirect pathways. It has been traditionally thought that the three pathways are involved in different aspects of controlling action, with direct pathway facilitating, indirect pathway inhibiting, and hyperdirect pathway stopping movements. Particularly, it has been hypothesized that the hyperdirect pathway, beginning at the subthalamic nucleus (STN), and the direct pathway, originating from D1-expressing striatal projection neurons, undergo a competitive 'race' during action stopping, depending on the relative timing of opponent signals from these two pathways arriving at substantia nigra pars reticulata (SNr), the output nuclei of basal ganglia. However, this view of isolated, different parallel pathways competing for single behavioral outcome is somehow limited, due to the fact that there are multiple feedback circuits that exist in the circuitry, implying a large role for crosstalk between pathways. Therefore, we explored the possibility that hyperdirect pathway can interact with direct and indirect pathways by regulating dopamine release in the dorsal striatum. To investigate this, we utilized optogenetic tools to drive high-frequency firing of STN neurons and simultaneously recorded subsecond changes in dopamine concentrations in the dorsal striatum using fast-scan cyclic voltammetry. Interestingly, irrespective of the multiple possible neural pathways from STN to substantia nigra dopamine neurons, selective blue-laser activation of STN neurons resulted in phasic increases in dopamine concentration in the dorsal striatum, with amplitudes resembling those of dopamine signals recorded during motivational events and behavior. Additionally, to determine behavioral effects of STN stimulation, mice were chronically implanted with fiber optics capable of delivering laser light during locomotor behavior and action learning. Indeed, high-frequency optogenetic stimulation of STN inhibited locomotor activity, consistent with the traditional model of its role in action stopping. However, surprisingly it was found that optogenetic STN stimulation could differentially control operant behavior and learning, which may be at least partially mediated through regulation of striatal dopamine. Our results thus indicate that the STN and hyperdirect pathway could affect the striatal direct and indirect pathways, and they may play a much more complex role in action control than previously thought.

Disclosures: C.D. Howard: None. **J.R. Klug:** None. **X. Jin:** None.

Poster

442. Systems Physiology and Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 442.15/KK4

Topic: D.15. Basal Ganglia

Title: Differential modulation of striatal neurons by brainstem cholinergic afferents

Authors: *J. MENA-SEGOVIA[1], I. HUERTA-OCAMPO[1], P. BOLAM[1], T. GERDJIKOV[2], D. DAUTAN[1,2]
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Abstract: Acetylcholine plays a major role in the modulation of neurons and microcircuits of the striatal complex. We recently reported that in addition to local cholinergic interneurons, a major source of striatal acetylcholine are the pedunculopontine (PPN) and laterodorsal tegmental (LDT) nuclei which provide a direct cholinergic innervation over different regions of the dorsolateral striatum and nucleus accumbens. The projections are topographically organized and to give rise to collaterals innervating the thalamus and the dopaminergic midbrain. In contrast to the local cholinergic synapses, which are predominantly symmetric, the synapses formed by brainstem axons are mainly asymmetric. These findings raise the question as to whether the cholinergic afferents from the brainstem play a distinct modulatory role to that of the local cholinergic neurons. To address this issue, we drove the expression of channelrhodopsin-2 under the promoter of choline acetyltransferase in either brainstem neurons or striatal interneurons of ChAT::Cre+ transgenic rats. We used the *in vivo* juxtacellular method to record and label neurons in the striatum during the optogenetic stimulation of striatal or brainstem cholinergic axons. Subsequently, the molecular composition of the labelled striatal neurons was analysed using immunohistochemical techniques. Our results show distinct effects on different striatal neuronal subtypes: striatal projection (medium spiny) neurons show a consistent inhibition following stimulation of either striatal or brainstem cholinergic innervation. In addition, cholinergic interneurons showed an increased firing rate following the optogenetic drive of brainstem cholinergic axons, which contrasts with the notion of acetylcholine-mediated inhibition of cholinergic interneurons. Our data suggest differential, but presumably complementary, roles of brainstem cholinergic afferents and local cholinergic innervation in the control of the activity of striatal neurons.

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Poster

442. Systems Physiology and Circuits

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Topic: D.15. Basal Ganglia

Support: NIH Grant MH101697

Title: Fast sensory responses in the pedunculopontine nucleus help pause actions

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Abstract: The ability to cancel planned actions when circumstances change is an important, yet poorly understood, aspect of self-control. The stop-signal task (SST) is a widely used paradigm in which GO cues direct actions and (in a subset of trials) a subsequent STOP cue instructs the subject to withhold action (i.e. override the GO command). In prior work (Schmidt et al. 2013 Nat Neuro) we discovered that neurons in the subthalamic nucleus (STN) rapidly respond to an auditory STOP cue, and appear to transiently pause the planned action by increasing activity in the substantia nigra pars reticulata (SNr). However there is still a missing link: how does STOP cue information flow into the STN? Previous studies have found neurons in the pedunculopontine nucleus (PPN) that respond to auditory cues with very short latency (~9ms), and PPN projects to STN among other targets. Thus, we hypothesized that the PPN provides fast STOP cue information to the STN. Specifically, we examined whether the cholinergic neurons of posterior PPN, thought to be important for sensory processing and attention, play a critical, temporally-specific role in stopping. We injected the Arch 3.0 virus (AAV-EF1a-DIO-eArch3.0-EYFP) bilaterally into the posterior part of PPN (AP: -8.2 mm, ML: 2.1 mm, DV: 6.3 mm) of ChAT::Cre rats. Optic fibers were implanted 300 μm higher than the injection sites, and test sessions started four weeks after the virus injection. We applied brief (150ms) pulses of yellow laser light (power: 20mW; duration: 150 ms) to hyperpolarize the cholinergic neurons on half of the trials, selected randomly. When the laser onset was 25 ms before the STOP cue, the rats' ability to stop was impaired (i.e. we observed a higher proportion of failed stop trials). This impairment was not seen if we shifted laser onset slightly, to 25 ms after the STOP cue, confirming that PPN cholinergic neurons have a rapid, time-limited function in STOP cue processing. Reaction time for GO trials was not significantly affected under either condition. We have also begun single unit recordings of PPN during the SST, and observed STOP cue responses of PPN neurons to the STOP cue with very short latency (~10 ms). Together our results support the hypothesis that the PPN-STN-SNr pathway provides a fast, transient mechanism for pausing behavior.

Disclosures: F. Chen: None. **J.D. Berke:** None. **R. Schmidt:** None. **N. Mallet:** None.

Poster

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Topic: D.15. Basal Ganglia

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Title: The globus pallidus cancels actions by suppressing striatal output

Authors: *R. SCHMIDT[1], N. MALLET[2], D. K. LEVENTHAL[3], F. CHEN[4], J. D. BERKE[4]
[1]Dept. of Biol., BrainLinks-BrainTools, Univ. of Freiburg, Freiburg, Germany; [2]Inst. of Neurodegenerative Dis., Univ. of Bordeaux, Bordeaux, France; [3]Dept. of Neurol., [4]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Behavioral inhibition is a fundamental component of executive function. In a standard test of behavioral inhibition (the Stop-signal task) subjects are given Go cues to prompt movements, but on some trials a subsequent Stop cue indicates that movement preparation should be cancelled. We previously showed (Schmidt et al. 2013, Nat Neuro) that individual neurons in the rat subthalamic nucleus (STN) respond very quickly to Stop cues (~15ms latencies), and on successful Stop trials this appears to drive excitation of neurons in the substantia nigra pars reticulata (SNr, ~35ms latencies) that would otherwise pause to allow movement initiation. However, we also found evidence that this is an incomplete account of action cancellation. The STN-SNr response is fast enough to explain the speed of stopping, but is highly transient and thus by itself may only delay action initiation. An additional slower, more selective mechanism likely plays a key role by suppressing movement-related activity within striatum. We hypothesized that this additional mechanism involves the globus pallidus (GP). We therefore compared the timing and selectivity of GP single-unit cue responses to other basal ganglia regions. We report that although GP cells exhibit heterogeneous firing patterns, their overall responses to Stop signals are slower (~60-100ms) yet more selective than STN and SNr cells. Furthermore, the time course of GP Stop responses closely matches the time when movement-related activity in striatum is suppressed, in line with pallidostriatal inhibition. As pallidostriatal inhibition is primarily mediated by arkypallidal GP neurons (Mallet et al., 2012, Neuron), we categorized our GP neurons into putative arkypallidal and prototypical cells based on electrophysiological features during behavior and sleep. Preliminary analyses suggest that the

slow, selective stop-signal responses are more pronounced in arkypallidal than in prototypical GP units. We conclude that movement cancellation involves multiple circuit-level mechanisms within the basal ganglia, with complementary temporal profiles and selectivity.

Disclosures: R. Schmidt: None. **N. Mallet:** None. **D.K. Leventhal:** None. **F. Chen:** None. **J.D. Berke:** None.

Poster

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University of Michigan

Title: Dynamic delta-beta phase-amplitude coupling predicts behavioral performance in a rat stop-signal task

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Abstract: Basal ganglia (BG) oscillations are associated with distinct behavioral states and pathologic conditions. Specifically, “beta” (~15-30 Hz) power is correlated with bradykinesia and rigidity in Parkinson Disease. We have previously shown (Leventhal et al, Neuron, 2012) that transient bursts of coordinated BG-wide beta oscillations occur after healthy rats utilize external cues to determine a motor plan. Furthermore, beta oscillatory power predicts Go reaction time (RT). Here, we extend these observations by analyzing dynamic oscillatory phase and power across a wide frequency range. Data from our previous report were re-examined. Male Long-Evans rats were trained to perform a stop-signal task, with electrodes implanted in

the striatum, globus pallidus (GP), substantia nigra pars reticulata (SNr), and subthalamic nucleus (STN). The pitch of a pure tone instructed rats which direction to move ("Go" trials). On a subset of trials, a subsequent white noise burst indicated that the rats should arrest their planned movement ("Stop" trials). Local field potentials were filtered in narrow bands from 1 to 100 Hz, and Hilbert transformed to provide continuous measures of phase and power. We found that within this frequency range, only beta power predicts RT. However, beta power is modulated by the phase of ongoing delta (2-4 Hz) oscillations, and so delta phase also predicts RT. In addition, the phase of delta oscillations preceding the STOP signal predicts whether stopping will be successful. These results show that the previously reported cross-frequency coupling in BG circuits occurs dynamically at critical moments of behavioral performance. The neural mechanisms underlying the regulation of beta power by delta phase remain to be elucidated, but may provide critical insight into mechanisms of response variability.

Disclosures: D.K. Leventhal: None. **J.D. Berke:** None. **J.R. Pettibone:** None.

Poster

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Topic: D.15. Basal Ganglia

Support: F32NS082043

2R56NS045130

Title: Dynamics of cortical and striatal activity during vibrissa-CS trace eyeblink conditioning

Authors: *C. A. THORN, E. MCDONNELL, C. MOORE
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Abstract: Cortico-basal ganglia loop circuitry is critical for certain types of associative learning, including stimulus-response (S-R) reinforcement-based learning. Plasticity in the cortico-striatal network is correlated with improvements in behavioral performance in a variety of S-R learning tasks. We aimed to characterize the learning-related changes that occur in cortical and striatal projection neurons during one such task - a trace-eyeblink paradigm shown to depend on intact forebrain circuits, including primary somatosensory cortex (SI) and striatum (Galvez et al., 2007; Flores & Disterhoft, 2009). To support these studies, we have developed an extended-trace

eyeblink paradigm for head-fixed mice. Vibrissal stimulation served as the conditioned stimulus (CS), corneal airpuff as the unconditioned stimulus (US), and trace intervals up to 2 seconds were used. Mice improved their performance on the task with over several days of training, as assessed by an increase in the percentage of conditioned responses made following CS presentation. Preliminary electrophysiology recordings were made in SI and striatum from mice performing the extended-trace eyeblink task. Neurons in both regions respond with short latencies to both vibrissa CS presentation and airpuff US presentation. Task-related dynamics in spiking and local field potential recordings were observed in cortical and striatal regions across task performance.

Disclosures: C.A. Thorn: None. **E. McDonnell:** None. **C. Moore:** None.

Poster

442. Systems Physiology and Circuits

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Topic: D.15. Basal Ganglia

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Title: Context-dependence of action selection in the basal ganglia

Authors: *M. LINTZ, G. FELSEN
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Abstract: Movements are controlled by an interconnected network of brain regions, but how activity in these different regions contribute to the stages of motor control - from action selection through outcome monitoring - is poorly understood. In particular, whether movements that are cued by external stimuli and internally generated movements are subserved by the same circuits is unknown. The basal ganglia (BG) and midbrain are known to be involved in motor control, particularly for directional orienting movements. As one of the two output nuclei of the BG, the substantia nigra pars reticulata (SNr) provides an excellent target for studying how BG output to downstream motor targets controls movements. The classic “disinhibition model” holds that the SNr provides tonic inhibition to these targets, and phasic release from inhibition allows for movement initiation. However, this model overlooks the diverse firing patterns subsequently

observed in the SNr and is thus incomplete. Here we examine how the SNr contributes to a critical aspect of motor control: the selection of one action from among alternatives. We recorded extracellular, single unit activity from the SNr of C57BL/6 mice performing a novel odor-cued spatial choice task comprised of interleaved blocks of "choice" trials and "no-choice" trials. In choice trials, animals sampled a binary odor mixture and decided whether to move to the left or right reward port based on the dominant component of the mixture. In no-choice trials, the odor mixture always consisted of equal concentrations of both components, and reward was available at the same side on every trial of the block. This task allows us to compare SNr activity preceding movements that are either stimulus-cued (choice-trials) or internally-generated (no-choice trials), but which are otherwise identical. Mice perform well on this task, exhibiting unbiased behavior during choice blocks and reliably returning to the rewarded port during no-choice blocks. Consistent with previous studies we found that, preceding movement, the firing rates of subpopulations of SNr neurons depended on the direction of upcoming movement. However, we also found that activity depended on the context in which the movement was selected (i.e., in choice vs no-choice trials). These results suggest that the BG may differentially mediate the selection of stimulus-cued and internally-generated actions.

Disclosures: M. Lintz: None. **G. Felsen:** None.

Poster

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Topic: D.15. Basal Ganglia

Support: CIHR

Title: Discharge characteristics of striatal neurons during visually guided locomotion in the cat

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Abstract: Successful adaptation of locomotion according to changes in the environment requires that the central nervous system (CNS) use visual information to plan the necessary anticipatory modifications of gait. Previous studies have demonstrated an important contribution of the motor cortex (area 4) and the posterior parietal cortex (PPC, area 5), respectively, to the execution and the planning of such gait modifications (Drew et al. 2008). The locomotor deficits observed in

Parkinson's disease equally suggest an important contribution of the basal ganglia in the control of locomotion. Yet, we are lacking a fundamental understanding of basal ganglia function at the single cell level for this motor behavior. The present study addresses this issue by presenting preliminary data on the discharge characteristics of striatal neurons during unobstructed treadmill locomotion as well as during voluntary gait modifications in intact, unrestrained cats. Single unit activity was recorded from cells in the caudate and putamen as well as in the striatal bridges between the two. Muscle activation patterns from selected fore and hindlimb flexor and extensor muscles were recorded simultaneously. Cells with phasic modulation of their discharge activity were observed in all three regions. As in the motor cortex, most cells were characterized by a phasic discharge pattern occurring once every step cycle, phase-linked to the activity of the contralateral fore- or hindlimb muscles. Most striatal cells discharged throughout either the swing or stance period of the limb, with little evidence of fractionation of the pattern as observed in motor cortical cells. (Drew et al. 2008). In addition, most modulated cells showed no, or only relatively small, changes in discharge frequency during the steps over the obstacle. Again, this is in contrast to the large changes in discharge frequency observed in motor cortical cells. A small population of cells showed changes in activity prior to the step over the obstacle of the type observed in the PPC. These results are more compatible with a general contribution of the basal ganglia to the regulation of step cycle duration and cadence than to a more specific contribution to the selection of the muscle activation patterns required to modify gait. Drew T, Andujar J-E, Lajoie K, Yakovenko S. Cortical mechanisms involved in visuomotor coordination during precision walking. Brain Res Rev 57: 199-211, 2008.

Disclosures: **J.A. Leonard:** None. **I. Arto:** None. **T. Drew:** None.

Poster

442. Systems Physiology and Circuits

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Title: Pallidal and cerebellar control of thalamocortical activity

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Abstract: Both the basal ganglia and cerebellum receive inputs from the cerebral cortices and project back to the original cortices via the thalamus, and control voluntary movements. Thus, for elucidating roles of these structures on voluntary movements, it is crucial to understand how their outputs control thalamocortical activity. In the present study, we examined effects of outputs from the basal ganglia and cerebellum on activity of thalamocortical neurons projecting to the motor cortices of macaque monkeys under awake states. Thalamocortical neurons were identified by antidromic responses to stimulation of the primary motor cortex and supplementary motor area. Then, the responses of the thalamocortical neurons to stimulation of the cerebellar nucleus (CN) and the internal segment of the globus pallidus (GPi), which are major output nuclei of the cerebellum and basal ganglia, respectively, were examined. Thalamocortical neurons receiving CN inputs were found in the posterior part of the motor thalamus, while those with GPi inputs were located in the anterior part, and only a few neurons responded to both CN and GPi stimulation. Single pulse stimulation of the CN evoked biphasic responses composed of short latency brief excitation followed by inhibition. During repetitive CN stimulation at 50 or 100 Hz, a train of biphasic responses corresponding to each stimulus pulse, was observed. On the other hand, single pulse stimulation of the GPi evoked inhibition, which was often followed by subsequent firings. During repetitive GPi stimulation at 50 or 100 Hz, each stimulus pulse evoked short-latency inhibition and following firings, and the following firings occurred with higher probability and at more constant latency in the latter part of the stimulus train. After local injection of gabazine, GABA-A receptor antagonist, in the vicinity of recorded neurons, both the inhibition and following firings induced by GPi stimulation disappeared without significant changes of spontaneous activity. Thus the following firings can be considered as postinhibitory rebound excitation. These results suggest that cerebellum and basal ganglia outputs give differential effects on the thalamocortical activity: cerebellar outputs convey information to thalamocortical neurons through excitatory inputs immediately followed by inhibition, whereas basal ganglia outputs convey information through inhibitory inputs with rebound excitation.

Disclosures: **S. Chiken:** None. **Y. Kawaguchi:** None. **A. Nambu:** None. **M. Kimura:** None.

Poster

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Olle Engkvist Foundation

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Title: The role of the corticostriatal circuit in transitioning between motor behaviors

Authors: *M. J.-E. TAMTÈ, P. HALJE, P. PETERSSON
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Abstract: Although most motor acts are executed in a smooth uninterrupted manner, sets of identifiable movement subcomponents can nevertheless often be identified. In this view, complex motor acts can consequently be reduced to distinct combinations of simpler building blocks that are linked together into functional action chains. Although the neuronal substrate controlling the transitions between such subcomponents is unknown, the basal ganglia has attracted a particular interest as a possible node for concatenations of actions. To test this hypothesis we have here studied the neural activity of the corticostriatal system in relation to detailed aspects of rodent grooming - a natural, spontaneous and highly stereotypic sequential behavior. The grooming behavioral sequence is built up of four different motor programs executed in separate phases one after another, in varying order. If the corticostriatal circuits are indeed involved in controlling the switching between the different phases of the grooming sequence, specific neuronal modulations in relation to these transitions would be expected. Rats were bilaterally implanted with 64-channel multielectrode arrays centered on the forelimb area of the primary motor cortex and the corresponding parts of the dorsal striatum. Single units were isolated in the different recording channels in each structure and classified as being pyramidal cells, medium spiny neurons and cortical or striatal interneurons based on waveform characteristics and firing dynamics. Digital video recordings were used to identify the time points of behavioral switching events in the grooming sequences allowing for off-line temporal alignment of neuronal and behavioral data. Preliminary analyses show that a substantial fraction of neurons were significantly modulated during various phases of the grooming behavior and that some of these cells were specifically modulated during phase transitions. Firing rate modulations occur on a timescale preceding and/or succeeding the time point of the behavioral

transition with as much as half a second, indicating differential roles in behavioral switching. Further analyses will reveal if the recorded signals contain information relating to sequencing of longer action chains.

Disclosures: **M.J. Tamtè:** None. **P. Halje:** None. **P. Petersson:** None.

Poster

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Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, JST

Precursory Research for Embryonic Science and Technology,JST

Title: Behavioral and physiological impacts of aversive information in monkeys

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Abstract: Decision-making often comprises prediction of potential rewarding or punishing outcomes before a choice. However, the effect of aversive information on decision-making behavior and its neuronal mechanisms have not been well understood. To answer this question, we trained two monkeys (Macaca fascicularis) in a choice saccade task. In the task, three fractal images were separately associated with a rewarding juice (R), a neutral tone with a small reward (T), or an aversive airpuff (A). After fixating on a central point (FP), a pair of two images; R-T, R-A, or T-A, appeared in the left and right of FP. The monkeys then moved their eyes to one of the images to obtain a reward and/or to avoid a punishment. Both monkeys often showed deviation from the optimal choice in T-A trials, even after extensive training, supporting the complex interaction between opposing processes such as approaching to a salient stimulus and avoidance of an aversive outcome. To further examine the effect of the prospect of possible outcomes, one monkey was trained on the same task in a block design; the same image pair was repeated for 15-20 trials, while the side of the optimum image changed randomly and then the pair was changed unexpectedly. This allows the animal to predict the pair before its presentation except on the first trial of a block. We found that the inclusion of ‘A’ significantly affected the

behavior even when the optimum choice was identical; the rate of the optimum choice for R-A was lower than that for R-T. Reaction times were the shortest for R-A, while the variability was highest for T-A trials. The basal ganglia are thought to be involved in reward-based motor control, learning, and decision-making. Then, are there the neuronal mechanisms in the basal ganglia for the decision-making under the situation where punishments may occur, and if so, are they independent or integrated with the reward system? We found that 52 (43%) out of 122 task-related neurons in the primate caudate nucleus, an input channel of the basal ganglia, showed differential modulation in the pre-target fixation-period activity depending on the expected image pair. Among them, 28 neurons showed differential activity depending on the suboptimum image even the optimum image was the same (i.e. R-T vs. R-A), indicating the impact of the inclusion of an aversive image. Further, nearly half of them (13/28) did not discriminate R-A and T-A indicating that they are independent of reward circuits. These results indicate that a group of caudate neurons are affected by aversive information, sometimes independent of reward process.

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Poster

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Canada Research Chair Program (DPM)

Title: Functional connectivity of the subthalamic nucleus and substantia nigra pars reticulata changes during flexible action control

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Abstract: The subthalamic nucleus (STN) is a major input structure of the basal ganglia (BG) and a common hub for the two major BG inhibitory pathways (hyperdirect and indirect). Consequently, current models of BG voluntary motor control predict that STN efferent signals inhibit movement or incorrect motor plans. However, STN output can also facilitate movement,

via an opposing pathway to the substantia nigra pars reticulata (SNr, BG output structure) through the external segment of the globus pallidus. It is unclear how these conflicting signals from the STN contribute to voluntary motor control. Here, we compare the influence of the STN on goal-directed, and non-goal directed eye movement behaviour in two monkeys, to resolve whether STN output can vary between inhibitory and facilitatory effects according to behavioral condition. In the same monkeys, we compared the STN to the downstream SNr, which sends BG output signals to the thalamus and superior colliculus to influence saccades. We found that electrical stimulation of the STN (n = 41) inhibited eye movements in a goal directed task, but facilitated eye movements in a non-goal directed task, whereas SNr stimulation (n = 39) inhibited eye movements in both task conditions. Furthermore, simultaneous recording of local field potentials in the STN and SNr with electrode pairs (n = 15) revealed a high cross-correlation between structures at beta frequencies (14-30 Hz; associated with movement inhibition) during a presaccadic epoch in goal directed tasks, but at low gamma frequencies (35-60 Hz; associated with movement excitation) in the non-goal directed task. Using a simple Hodgkin-Huxley spiking model, we compared the probability of a saccade motor command reaching a threshold for movement initiation when preparatory signals exhibited either beta or low gamma frequencies. Using the same number of action potentials within a fixed time period, we found that a saccade motor command has a greater likelihood of reaching initiation threshold when preparatory signals exhibited low gamma frequencies compared to beta frequencies, due to increased oscillatory waveform amplitudes at higher frequencies. We suggest that when a rewarding goal is present, the STN increases inhibition from BG output to decrease unnecessary movements in favour of goal-directed movements. Alternatively, when no explicit goal exists, the STN reduces inhibition from BG output to facilitate automatic movements toward unexpected stimuli. This is accomplished by alternating between beta and low gamma oscillatory frequencies during movement preparation, which decreases and increases the gain, respectively, in nuclei critical for movement initiation.

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Poster

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Title: Overlapping cognitive and motor functions in the globus pallidus internal segment

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Abstract: The basal ganglia have been implicated in a variety of functions and tasks in motor control. To test the hypothesis that the basal ganglia are involved in facilitating desired behaviors and inhibiting competing ones, we recorded GPi neurons in two monkeys trained to perform a wrist movement task with four levels of potential competition: 1) turning on vs. turning off muscles, 2) movement toward an illuminated target (automatic) vs. movement away from that target (non-automatic), 3) suppression of movement in response to a cue (NoGo task), 4) performance according to over-learned rules vs. reverse rules. We then focally inactivated neurons in GPi with muscimol to determine the effects of inactivation on the motor and cognitive performance of the task. We recorded 98 GPi neurons from two monkeys that had activity changes related to some aspect of task performance. 52% of all recorded neurons encoded signals specifically related to the task type, rule, or their interaction. 82% of these cells modulated their firing rates prior to target presentation, mostly with increases in firing rate when the animals had to suppress prepotent responses. Another subset of GPi neurons (18%) signaled suppression of movement (NoGo), predominantly with an increase of firing rates. While most cells encoded just one task or rule-related parameter, 41% of all cells encoded two or more aspects. Muscimol (0.5 μg in 0.5 μl) was injected at 18 separate sites in the GPi of two monkeys where task-related neurons had been recorded. Inactivation caused substantial impairment of task performance that was reflected in: 1) overall increase of errors post inactivation, 2) increased impulsivity, 3) difficulty holding the cursor against the load (with loaded extensors), and 4) increased perseveration in task types requiring suppression of prepotent response. 23% of all injections impaired just rule aspect, 17% impaired task aspect, however the majority - 59% - affected both rule and task performance. These findings support the hypothesis that the basal ganglia are involved in action control at multiple levels, with an important role in suppression and facilitation of competing behaviors. Many neurons in the globus pallidus encode multiple aspects of the task, and inactivation of these cells impairs performance at multiple levels. Our results suggest that despite undeniable anatomical and physiological indication of parallel organization of functions in the basal ganglia, there is also clear evidence for integration of information and overlap of functions occurring in the basal ganglia at the level of the output nucleus, GPi.

Disclosures: **J.W. Mink:** F. Consulting Fees (e.g., advisory boards); Medtronic, Inc, Edison Pharmaceuticals, Inc. **I. Statnikova:** None.

Poster

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Robin and Richard Patton through the E. Bronson Ingram Chair of Neuroscience

Title: Comparative diffusion tractography of cortico-striatal motor pathways reveals differences between humans and macaques

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Abstract: The primate cortico-basal ganglia circuits have been understood to be segregated into parallel anatomically and functionally distinct loops. The motor loops of the basal ganglia have been described as a separate body movement loop with projections from primary motor cortex (M1) to the putamen, and an oculomotor loop with projections from the frontal eye fields (FEF) to the caudate nucleus. This classical textbook description of the circuitry has been guided by anatomical and physiological findings in macaques. However, functional and structural neuroimaging studies of the human cortico-striatal system show evidence inconsistent with this organization. In this study we used probabilistic diffusion tractography to conduct a direct comparison of the pattern of connectivity within these areas between humans and macaques. Nine adult macaques and nine healthy humans were scanned at a Phillips Achieva 3T MRI scanner, using similar pulse sequences and analysis pipelines for both species. In macaques we found that the FEF is connected with the head of the caudate and anterior putamen, consistent with previous neuroanatomical tract tracing findings. However, in humans FEF was connected to only a small portion of the caudate, with larger connections to portions of the posterior putamen. In both macaques and humans, M1 was connected with putamen, with termination zones overlapping with those of FEF in humans. These results show that in neither macaques nor

humans are the oculomotor and primary motor loops entirely segregated between caudate and putamen. Furthermore, the previous description of the anatomical connectivity of cortico-striatal motor systems in humans does not directly parallel that of macaques. This could be explained by the evolutionary expansion of prefrontal projections in humans. We do not think that this difference in gross anatomy between humans and macaques necessarily suggests a difference in circuitry between species.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Title: The cost of planning what movement trajectories will look like in extrinsic space: moving beyond the point-to-point reach

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Abstract: Motor planning is the process by which movement goals (e.g., target location) are transformed into motor commands. Recent work in our lab has suggested that motor planning of simple point-to-point reaches occurs almost instantaneously. That is, the majority of the reaction time (RT) is spent deciding upon the motor goal; once the target location has been identified, a motor command may be prepared with little additional reaction-time cost. It is unclear whether motor planning may be similarly rapid when the motor goal includes the specification of the movement trajectory: e.g., are any additional motor planning steps required to draw a letter “S”? Findings from bimanual interference tasks argue that motor planning may require a stage that represents the shape of the desired movement; generating congruent movements with both hands simultaneously is far easier than generating incongruent movements. We hypothesize that a resource-intensive (RT-consuming) stage may be invoked to plan how a movement will “look” in extrinsic space. In contrast, no additional RT should be consumed to subsequently execute such curved movements. To test this, a group of subjects reached between two targets while

avoiding virtual barriers - a task requiring subjects to plan movement trajectories with specific shapes that varied in complexity (i.e., curvature). We compared the RTs of these movements with those of a control group who were shown, in addition to the barriers, the shape of the trajectory to be generated. Hence, in the control group the movement path did not need to be planned, merely executed; furthermore, the execution requirements were identical in the two groups. We observed two striking results. First, the RT is consistently shorter for the control group across all barrier conditions, arguing that the execution of a curved movement in the absence of a planning requirement requires little additional RT beyond that needed to produce a simple point-to-point reach. Second, whereas the RT for the control group is fairly consistent across all barrier conditions, the RT in the test group fluctuates to a greater extent depending upon the complexity of the movement trajectory that must be planned. These findings argue that under certain task conditions, motor planning includes a stage that requires overt representation of the desired movement trajectory, and that this stage consumes a significant fraction of the total RT. We conjecture that it is this cognitive ability to explicitly represent abstract kinematics that has expanded the movement and tool-use repertoire in humans.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

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Title: Differential involuntary recruitment of arm muscles determine direction dependent haptic perception of uniform external perturbations

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Abstract: In this study, we investigated the shape of cost landscape for voluntary movements of left-handed and right-handed blindfolded human participants in response to lateral perturbations given to their hand. We computed four behavioral cost elements across three magnitudes of perturbations given to the left and right hand side. The asymmetry of the distribution of the normalized cost elements - rise time, best fit model order of the polynomial fitted to the instantaneous error of the human's position relation to a desired angle, steady state variability, and asymptotic stability - across leftward/rightward perturbations suggest that the subjects perceive the same perturbation magnitude differently depending on the direction of perturbation. Analysis of Electromyography (EMG) signals from eight arm muscles suggest that the pattern of involuntary muscle recruitment to stabilize the hand immediately after the perturbation is significantly different between leftward/rightward perturbations. Furthermore, there is no significant difference between cost landscapes for left/right handed groups. These observations suggest that haptic perception of external perturbations depends on involuntary muscle recruitment patterns to stabilize the hand.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Title: Readiness potentials of self-generated gait initiation

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Abstract: Introduction Gait initiation (GI) is an initial voluntary movement, required for the coordination of movement in the lower limbs. The activities of the supplementary motor area (SMA) and premotor cortex (PMC) are known to be important for the neural control of GI. Readiness potentials (RPs) are considered a potential variation reflecting the cortical activity of motion-related areas such as M1 and the SMA. However, only a few studies have analyzed their motion-related cortical activity in detail. Therefore, the aim of this study was to examine

activities of the SMA, PMC, and M1, at the time of GI, in detail. Methods The subjects were three healthy adult men (mean age: 26.7±1.2 years); informed consent was obtained from all participants. The subjects were asked to initiate gait for 30 trials. The GI was self-generated. All trials were initiated with a left footstep, and at least two alternating steps were executed. In the task, they were requested to gaze forward at the fixation spot placed 3m ahead in order to avoid blinks and eye movements. Electroencephalography (EEG) data were recorded continuously (bandpass, 0.01-70 Hz; sampling rate, 1024 Hz; ActiveTwo system, BioSemi, Netherlands) using 128 scalp electrodes positioned over the entire scalp according to the 10-20 system. Electromyograms (EMGs) were recorded along with EEG (bandpass, 10-510 Hz; sampling rate, 1024 Hz) using bipolar surface electrodes placed over the tibialis anterior muscle. The tibialis anterior muscle, which indicates the initiation of gait, was used as the trigger, and epochs were established 1500 ms before and 1000 ms after its onset. The baseline was derived from the average of the segment from 1500 to 1250 ms before the trigger point for each channel. The RPs were averaged over 30 trials. The averages RPs over all subjects were analyzed separately in left and right M1, PMC, and SMA area. Its statistical significance was assessed using the paired t-test. The level of significance was set at 5%. Results In the right hemisphere, the RPs occurred significantly earlier in the PMC ($p = 0.02$) and SMA ($p < 0.01$) compared to M1. There was a non-significant trend towards a longer latency in the SMA prolonged latency compared to the PMC ($p = 0.058$). Conclusions RPs latency indicated the timing of RPs rise. An extension of latency meant that the rise of RPs occurred early. Our results, confirmed that the SMA and PMC were activated prior to M1. These results indicated that the rise of RPs in the SMA is generated first in the right hemisphere responsible for the control of the swing of the lower limbs. This study suggests that the SMA may control the onset of muscle activity in self-generated GI.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Title: Strenuous exercise affects eye movements in the visual periphery

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Abstract: Musculoskeletal fatigue does not appear to significantly contribute to end-of-game injuries, thus other causes require investigation. We were interested in the extent to which cognitive decline, associated with strenuous exercise, leads to changes in motor control. We chose an activity (bicycle ergometry) and task (cued saccades) that would unequivocally separate muscle fatigue from motor performance to assess these relations. In this manner, we could monitor eye movement control independent of eye muscle fatigue. Nineteen physically fit individuals (19-28 y.o.) performed the experiment. Participants terminated cycling once they reached 90% maximum heart rate (MHR; 4 participants) or self-selected to terminate (15 participants; all achieved > 80% calculated MHR). The visual stimulus consisted of a centrally located Rapid Serial Visual Presentation in which six cue letters were presented, among distractor letters, to initiate a saccade to one of six peripheral targets. Cue letter / target mappings varied: an easy condition consisted of all target letters being mapped to one peripheral target location, and a hard condition consisted of each target letter being mapped to individual targets. Participants performed one block of each condition (counterbalanced) at each workload until they reached fatigue. Dependent measures included the ability to identify target letters within the central letter stream, initial saccade target selection, reaction time, first saccade endpoint accuracy and variability, peripheral dwell time, and the spatial extent of the dwell at the peripheral target. Our hypothesis was that as strenuous exercise persisted, participants would have greater difficulty in controlling eye movements to peripheral targets due to cognitive decline, and that this effect would be compounded in the hard task condition. We did not anticipate a change in visual perception with strenuous exercise, which was consistent with our results. Surprisingly, reaction times and initial saccade target selections did not change with strenuous exercise, although they were worse in the hard condition. Strenuous exercise had a transient effect on initial saccade endpoint accuracy in some participants. However, strenuous exercise largely affected peripheral dwells following the initial saccade. Although participants did not spend more time in the periphery when fatigued, the spatial extent of the peripheral dwells significantly increased. We interpret these findings as evidence of diminished eye movement control with strenuous exercise. Further study is required to determine the role of strenuous exercise on motor execution of other effectors.

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Poster

443. Cortical Planning of Actions

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EPSRC Prize Studentship

Title: The influence of transcranial alternating current on the initiation of movement

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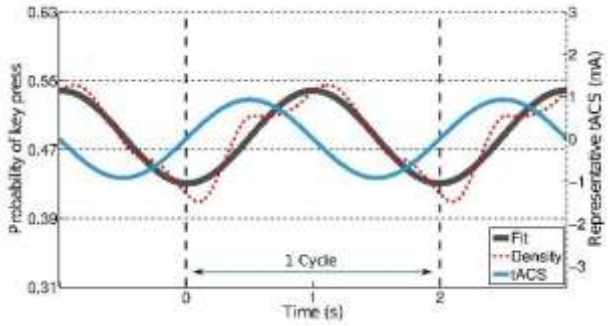
Abstract: The influence of the phase of transcranial alternating current stimulation (tACS) has been previously shown to modify behaviour; here we present an effect on the timing of self paced movement initiation that the subject is unaware of, and discuss potential explanatory models. Fourteen subjects performed a self-paced single finger key press task at a computer keyboard while exposed to tACS between 600uA and 925uA with a frequency of 0.5Hz, for a combined total of 700 minutes. Electrodes were placed within saline soaked sponges, with the reference electrode positioned over Oz, and the source electrode, placed over Fz. Key press times with reference to stimulation onset in the stimulation condition were collected and converted to equivalent phases corresponding to the relative phase of stimulation. In the stimulation condition, key press phases display a significant non-uniformity (Rayleigh test, $N = 2636$, $p = 0.024$). Furthermore, upon exclusion of two subjects with the lowest variance in waiting times and high key press rate, a strong statistical significance is reached in the stimulation condition (Rayleigh test, $N = 2121$, $p = 0.001$). Non-parametric density estimation of key press phases show a cyclical variation in probability matching a phase shifted stimulation waveform (see figure - red: density estimation, black: sinusoidal fit to density estimation, blue: representative tACS stimulation waveform). We hypothesise that the interaction between tACS and the neuronal activities of the subject result in the modulation of their decision making process. We address this question by augmenting models of decision making with the expected influence of non invasive brain stimulation, by testing model predictions concerning reaction times under non invasive stimulation experimentally, as well as by directly measuring the modulated brain activity using EEG.



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Title: The effect of coordinate frame on motor learning in Alzheimer's disease

Authors: ***D. PRESS**[1], Y. R. MIYAMOTO[2], J. M. BRETON[1], J. B. BRAYANOV[2], M. A. SMITH[2]
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Abstract: Alzheimer's disease (AD) causes apraxia, an impairment in performing previously learned motor skills, but its effects on the acquisition of new motor skills has been variable in different studies. Distinct neural systems allow for acquisition of motor skills in an intrinsic or body-based reference frame as compared to an extrinsic, world-based reference frame. The mixed results from previous studies may reflect this dichotomy. The neuropathology of AD shows prominent dysfunction in parietal regions that may be critical for acquisition of extrinsically-referenced skill, but there is relative sparing of motor cortical regions likely critical for the acquisition of intrinsically-referenced skill. We therefore hypothesized that AD participants will display a deficit in extrinsically-referenced learning leading to a shift towards acquiring skill in a more purely intrinsic reference frame. We recently showed that healthy control participants learn a visuomotor rotation (VMR) task in a combination of intrinsic and

extrinsic space. We thus tested AD participants (n=14) and similarly aged controls (n=6) on this task in which participants were trained for 120 trials to adapt 9cm point-to-point reaching arm movements to ±30° VMR for a single target location in one workspace. The generalization of this adaptation across 10 different movement directions was then tested both in the trained workspace and a novel workspace, which required the arm posture to be rotated by 90°. This change in arm posture results in a 90° shift in the correspondence between intrinsic and extrinsic representations of the movement direction. The extent to which the peak generalization shifts away from the trained target in the novel workspace can be used to quantify the extent to which the learned adaptation is represented in intrinsic (body-based) vs extrinsic (world-based) reference frames. No shift would occur if adaptation were represented in a purely extrinsic frame, because the extrinsic movement direction corresponding to the trained target is maintained, while a full 90° shift in generalization would be expected if adaptation were represented in a purely intrinsic frame. In the novel workspace, we observed shifts in generalization that were nominally closer to 90° for the AD participants than for the healthy elderly controls (72±6° vs. 59±7°), in line with reduced extrinsically-referenced learning in AD, but the results did not yet reach statistical significance ($p = 0.08$). These preliminary results suggest that AD participants can learn a visuomotor rotation task but that extrinsically-referenced learning may be impaired, perhaps related to parietal lobe dysfunction.

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Poster

443. Cortical Planning of Actions

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Program#/Poster#: 443.07/KK23

Topic: D.17. Voluntary Movements

Support: LLU Physical Therapy Department

NIH Grant (T32 HD064578)

Title: Brain activation associated with decoupling muscle synergies of the human pelvic floor

Authors: *S. ASAVASOPON[1], M. RANA[2], D. J. KIRAGES[2], M. S. YANI[2], E. B. LOHMAN[1], L. S. BERK[1], J. J. KUTCH[2]
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Abstract: Human pelvic floor muscles have been shown to operate synergistically with a wide variety of muscles, which has been suggested to be an important contributor to continence and pelvic stability during functional tasks. We have recently identified that pelvic floor muscle (PFM) synergies are facilitated by motor cortical regions controlling the PFM. Here we investigated whether or not PFM synergies are intrinsic, or can be modified by training participants to decouple PFM from synergistic muscles. We hypothesized that, if participants could voluntarily decouple a PFM synergy, they would do so by altering activity in motor cortical regions controlling PFM. In the current study, 20 healthy males performed two types of gluteus maximus muscle (GMM) tasks described as being coupled or decoupled to the PFM. In the coupled condition, we instructed participants to activate the GMM, which is naturally accompanied by a synergistic activation of the PFM. In the decoupled condition, we instructed participants to activate the GMM while maintaining a relaxed state of the PFM (which was facilitated by providing electromyographic (EMG) feedback of activity in the PFM). We found, using EMG recordings from the PFM and GMM, that participants had significantly lower correlation between PFM and GMM activity during the decoupled condition compared to the coupled condition ($p<0.05$). We then examined a subset of participants during the coupled and decoupled conditions using functional magnetic resonance imaging (fMRI). Our main finding was that the execution of the decoupled task as compared to the coupled task activated the anterior cingulate cortex (ACC) and left anterior insula ($Z>2.3$; $p<0.05$; cluster corrected), but did not support the hypothesis of altered motor cortical activation in regions associated with PFM activity at the same significance level. We interpret our findings to suggest that muscle synergies of the PFM are relatively intrinsic, and are not modified by adjusting activity in primary motor cortical areas. Our results suggest that the ACC may employ a modulatory effect on the primary motor cortex and the supplementary motor area, which facilitates the suppression of PFM muscle synergies. In addition, our findings suggest that the left anterior insula may mediate somatic and visceral attention to the interoceptive state of feeling PFM relaxation. Complex motor tasks that require awareness, training, and focus on intricate somatic and visceral areas such as the pelvic floor complex may require participation of the brain regions associated with interoception as well as motor control. We thank Darryl Hwang for assistance with the fMRI protocol.

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Poster

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Support: Department of Biokinesiology and Physical Therapy University of Southern California

Department of Physical Therapy Loma Lina University

T32 HD064578

Title: Cortical-facilitated muscle synergies of the human pelvic floor

Authors: M. RANA[1], S. ASAVASOPON[2], D. J. KIRAGES[1], M. S. YANI[1], B. E. FISHER[1], E. B. LOHMAN[2], L. BERK[2], *J. J. KUTCH[1]
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Abstract: Human pelvic floor musculature is made up of several skeletal muscles and is an important contributor to continence and functional pelvic stability during functional tasks. Synergistic pelvic floor muscle (PFM) activity has been shown to occur in advance of activity in the primary muscles used to complete many postural tasks, suggesting that pelvic floor muscles may be part of a feedforward synergy used to perform an anticipatory postural adjustment (APA). Despite the potential relevance of pelvic floor synergies in prevalent clinical conditions including incontinence and chronic pelvic pain disorders, the neural mechanism of pelvic floor synergies remains unknown. Since extensive research has demonstrated the cortical underpinnings of APAs we hypothesized that PFM synergies may be facilitated by activity in specific motor cortical areas that contribute toward PFM contraction. 14 healthy men were recruited for three experimental sessions. In the first session electromyographic (EMG) recordings were obtained to characterize the synergistic action between PFM, gluteal muscles (Glut) and the first dorsal interosseous (FDI). The second session involved functional magnetic resonance imaging (fMRI) to quantify the cortical activation during voluntary activation of the three muscles. The final session involved transcranial magnetic stimulation (TMS) over an fMRI-identified region of interest to verify fMRI findings. All participants could perform isolated voluntary PFM and FDI contractions, but voluntary Glut contractions were accompanied by significant ($p<0.05$) pelvic floor activation. fMRI results showed that a region of the medial wall of the precentral gyrus was consistently activated ($z>2.3$; $p<0.05$; cluster corrected) during both voluntary PFM contractions and Glut contractions but not during voluntary FDI contractions. Application of TMS over the medial wall of motor cortical region, where significant activity occurred during both voluntary pelvic floor contraction and gluteal contraction, elicited significant ($p<0.05$) motor evoked potentials (MEP) in the pelvic floor. Since the region we identified in the medial wall of precentral gyrus is active during pelvic floor contraction, during which the gluteal muscles are not active, we believe it to be related to pelvic muscle contraction. This was further confirmed by the generation of pelvic floor MEPs from this region. Thus, muscle synergies of the human pelvic floor appear to be facilitated by the activation of motor cortical areas. We thank Dr. Darryl Hwang and Dr. Ya-Yun Lee for assistance with the fMRI and TMS protocols.

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Poster

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Title: Evidence that an autonomic 'central command' originates in cortical motor areas

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Abstract: Previously, we identified 7 cortical motor areas in the frontal lobe of monkeys that are viewed as generating central commands for voluntary movement. Visceral autonomic arousal precedes the initiation of physical stressors such as exercise and postural changes. This predictive autonomic regulation is also thought to be due to a central command. To determine a potential origin of the autonomic central command, we used retrograde transneuronal transport of rabies virus to define the cortical areas with the most direct influence over the adrenal medulla in Cebus monkeys. Remarkably, we found that each of the 7 motor areas in the frontal lobe is synaptically linked to the adrenal medulla. These areas include the rostral, dorsal and ventral cingulate motor areas and the supplementary motor area (SMA) on the medial wall and the

dorsal premotor area (PMd), ventral premotor area and primary motor cortex (M1) on the lateral surface. All of these motor areas are interconnected and project directly to spinal cord. Cortical projections from the motor areas to the adrenal medulla were initially found in layer V after 3rd order transneuronal transport, i.e. disynaptic connections to sympathetic preganglionic neurons in the thoracic spinal cord. As transneuronal transport progressed to label 4th, 5th and 6th order neurons in separate animals, the number of labeled neurons in the motor areas increased dramatically. After 6 orders of transport, M1 had the largest number of labeled neurons of any cortical area. Collectively, the number of labeled neurons in the motor areas was greater than in each of the other cortical systems most frequently associated with autonomic control (e.g. limbic, anterior cingulate, medial prefrontal, insular, orbitofrontal). Based on the motor map of the body in M1, its projections to the adrenal medulla are localized within its trunk representation. Similarly, PMd and SMA projections appear to originate from their trunk representations. Overall, these observations suggest that the central command responsible for predictive changes in autonomic function originates from multiple cortical motor areas. The co-localization of descending control of the adrenal medulla in the cortical motor areas suggests that skeletomotor and autonomic control may be integrated at the cortical level. In particular, this co-localization within the trunk representation suggests that exercises targeting core body musculature such as yoga and pilates may be beneficial for regulating the sympathetic nervous system. Furthermore, dual control within each motor area may contribute, in part, to the disruption of autonomic regulation observed in Parkinson's disease.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Title: Saccade planning activity dissociated from visual attention activity in human parietal cortex

Authors: *W. E. HUDDLESTON, J. R. LYTLE, M. S. ALEKSANDROWICZ
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Abstract: Regions in parietal cortex have been implicated in saccade planning, although the extent to which this brain region is involved in motor planning versus visual attention components of the task have previously not been well dissociated. The purpose of this study was to identify and characterize regions of parietal cortex involved in planning visually guided saccades while tightly controlling the focus of visual attention. Ten healthy participants (18-43 years, 4 males, 1 left-handed) performed a cued saccade task while undergoing functional magnetic resonance imaging (fMRI). The stimulus consisted of a central rapid serial visual presentation (RSVP) of letters. Four peripheral targets also had an RSVP string of letters present at all times. During each trial, participants received two cues embedded within the centrally located RSVP. The target cue was presented first and indicated the location of the target. The 'go' cue was presented after a variable delay of 2 or 4 seconds, instructing the participant to perform a saccade to the correct target. During the delay, visual attention was focused centrally on the RSVP while the participants monitored the letter stream for the 'go' cue, and the focus of motor intention was at the cued peripheral target. Eye movement data were collected at 120 Hz in the 3T MRI scanner (GE; Milwaukee, WI) using a remote optics bright pupil camera system (ASL; Bedford, MA). Overall, the topographic maps of saccade targets were quite specific bilaterally, with 51% of voxels in the left map and 60% of voxels in the right map active for only one specific target location. In a control experiment using the same stimulus, we identified parietal regions involved in a purely visual attention task for comparison. Behavioral performance on the two tasks was not significantly different ($p = .378$), yet the extent of activation in parietal cortex was significantly greater for the visual attention experiment ($p = .004$). That said, only 21% of the motor-intention-specific voxels in both hemispheres overlapped with the visual attention maps. The orientation of the motor intention maps and visual attention maps varied significantly among participants. The results of the 2 experiments demonstrate that motor intention maps can be dissociated from visual attention maps in parietal cortex and that both maps are spatially specific. No consistent orientation of these maps across people existed. Rather, these participant-specific differences in parietal cortex may reflect alternate strategies used during the task and provide further insight into the relations and dynamic functional organization of human visual and motor attentional processes.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Support: ZNZ Fellow

Title: Changing resting state connectivity measured by functional magnetic resonance imaging with transcranial alternating current stimulation

Authors: ***M. T. BÄCHINGER**[1], M. MOISA[2], R. POLANIA[2], D. MANTINI[1,3], C. RUFF[2], N. WENDEROTH[1]
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Abstract: Transcranial alternating current stimulation (tACS) is a non-invasive technique in which sinusoidal electrical currents are applied to the brain. Recent studies have proposed that tACS is capable of modulating oscillatory brain activity - as for example measured with electroencephalography (EEG) - in a frequency- and phase-specific specific way. These neuronal oscillations are also correlated with the blood-oxygen-level dependent (BOLD) signal measured with functional magnetic resonance imaging (fMRI). Here we investigate whether it is possible to enhance resting-state connectivity by applying tACS over the motor cortex in the individually-defined alpha band (8-12 Hz; the predominant frequency band observed over the motor cortex in EEG during rest). In a first step, we measured EEG over the two motor cortices during rest (with open eyes). From these data, we calculated the individual alpha peak over the motor areas, as well as the phase-relationship of the two signals and their envelopes. In a second step, we used tACS to "play-back" the individual alpha-frequency signal. Two electrodes were placed over the motor cortices of the left and right hemisphere and a third reference electrode was placed over the inion. Two different conditions were tested: In the first, the two signals and their envelopes reflected the same phase-relationship as measured during EEG whereas in the second, the phase-relationship of the two signals and envelopes was inverted. Before, during and after stimulation we measured resting-state fMRI. To our knowledge, this is the first time that a three-electrode tACS setup was used with concurrent fMRI measurements. We assessed interhemispheric connectivity between the two motor cortices using a seed-based approach. Left and right M1 were localized by overlaying an anatomical mask defined by functional activity measured during a simple motor task. Using left or right M1 as seed region, we analyzed how resting state fMRI connectivity changed within as well as across hemispheres. Our preliminary results show that applying tACS that mimics the physiological phase-relationship of the two alpha bands and its envelopes increases BOLD connectivity within the sensorimotor network during and after stimulation. By contrast, stimulation with inverted signals (phase shifted by 180°) decreases BOLD connectivity. Our results suggest that tACS with appropriate stimulation parameters can selectively modulate BOLD connectivity of functionally specialized networks in the human brain.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Title: Cortico-muscular network dependent on handedness and perspective during action recognition: Towards a neurophysiological model of action simulation

Authors: ***R. KELLY**, L. A. WHEATON
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Abstract: Investigating the neurophysiology behind our action encoding system is a way to investigate the underlying mechanisms regarding how we understand seen action. Previous research suggests that seen actions elicit the same action in the observer's motor system even when the seen action is not executed. Additionally, it has been shown that when observing an action, the observer's motor system directly matched the action onto their own motor system. The purpose of this study is to evaluate the interaction of handedness and perspective and how it plays a role in our ability to understand an action. Right-handed subjects were presented with randomly organized static visual images of tools, from either an egocentric or allocentric perspective, being used by either a left or right hand while squeezing hand dynamometers. EMG electrodes were placed on both arms and recorded from the flexor/extensor and the pronator teres muscle. Simultaneously we recorded electroencephalography (EEG) from the C3 and C4 electrodes in order to observe hemispheric differences over the motor areas. Subjects were asked to judge what was occurring in the image and respond by pressing a button to obtain accuracy and latency data. Our hypothesis was that right-handed subjects would predict action best from an egocentric perspective with subjects mapping the seen action onto the matching limb (limb match condition). In the allocentric perspective, subjects would map the seen action as if they were looking in a mirror (mirror matched condition). The results showed that there is a matching pattern of muscle activation that corresponds with neural laterality patterns in the Mu band (10-12 Hz). Results suggest a cortico-muscular network exists to confirm our hypothesis. This finding demonstrates that seen actions are encoded based on perspective, demonstrating a distributed pattern of how actions are mapped along the neuraxis. These networks are dependent

on which limb, dominant or non-dominant, is seen. This study will add to our basic knowledge of the motor simulation process and can generalize to populations with disorders affecting the neural control of movement.

Disclosures: R. Kelly: None. **L.A. Wheaton:** None.

Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Support: Grant-in-Aid for Young Scientists (B)

The National Institute of Information and Communications Technology

Title: Transmitting signals from premotor and primary somatosensory cortices construct muscle-like coordinate representation in the primary motor cortex

Authors: *Y. FUJIWARA[1], W. YASUDA[1], J. LEE[2], T. ISHIKAWA[2], S. KAKEI[2], J. IZAWA[3]
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Abstract: Making a movement toward a goal, the brain computes transformations from the coordinate frames of sensory inputs (extrinsic) to the coordinate frames of muscles (intrinsic). A lot of neurophysiological studies illustrated several coordinate frames (e.g., retinotopic, muscle and proprioceptive) in the parietal and the frontal cortices. Nevertheless, it has not been explored yet how the brain implements the sensorimotor transformation by integrating different kinds of information processed in these areas. We approach this question by presenting a novel method analyzing fMRI data for investigating a voxel-level functional connectivity between multiple cortical areas. Specifically, we focused on how the motor related activities in the primary motor cortex (M1) were computed as a result of integration of signals from other cortical areas such as ventral premotor (PMv), dorsal premotor (PMd) and primary somatosensory cortices (S1) when the participants performed isometric torque generation task of the wrist. We adopted an established experimental paradigm for monkey neurophysiology developed by Kakei et al. for a neurophysiological study using primates (1999) and extended it for a human experiment where the participants made isometric force toward eight target directions with two different forearm

postures while intramuscular electromyography (EMG) and fMRI were measured. The preferred directions of the wrist muscles rotated about halfway of the forearm rotation when the subjects changed their forearm from pronated to mid posture (90 degree supination from pronated posture). This result indicates that the activities of the human wrist muscles are in the intermediate coordinate frame between the extrinsic and the joint coordinates, which was congruent with the previous monkey study (Kakei et al. 1999). Then, fMRI decoding analysis showed that M1, PMv and S1 activity patterns were in the range of the muscle coordinate while PMd was in the range of the extrinsic coordinate. To explore inter-areal integration, we predicted the M1 voxel patterns from a combination of PMv and S1 voxel patterns. Importantly, the predicted M1 activities preserved the muscle-like coordinate that was observed in the actual M1 activities. This indicates that integrating signals in PMv and S1 might effectively construct information represented in M1. We suggest that integrity of M1 muscle-like coordinate is a result of inputs from both the sensorimotor transformation represented in PMv and the sensory feedback represented in S1. The newly developed fMRI voxel-wise analysis shed light on a hidden process of coordinate transformation mediated by multiple cortical areas.

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Poster

443. Cortical Planning of Actions

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Topic: D.17. Voluntary Movements

Support: KAKENHI 20508783

Title: Voluntary stopping of ongoing behavior relates to the inferior parietal activation

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Abstract: Purpose: Both internal and external factors modify our behaviors in our daily lives. A general belief might be that our will per se controls our behaviors. Hence, imaging studies on which brain areas subserve voluntary movements have been fascinating many researchers.

Despite many challenges on the neural correlates of volition and intention, brain regions associated with voluntary stopping of ongoing behaviors has not been well understood. In this study, we focused on the brain activity of difference between voluntary and passive behaviors and an effect of thinking on behavioral changes. To investigate the above brain activities at the time of a behavioral change induced voluntarily and passively, we conducted a finger-tapping task using the functional magnetic resonance imaging (fMRI). Here, we report some results of the pilot study. Methods: Five healthy volunteers underwent 3T MRI scans (Philips, Ingenia). A short block design was employed with four conditions: voluntarily and passively cued initiation and suppression of finger tapping. Each condition was presented 6 times randomly in a session. All subjects performed three sessions. In each block, a white cross was basically presented for eyes fixation, and just after 'Self' or 'Cue' word presentation, a few hundred milliseconds lasting green and red cross were shortly presented as signals for initiating and stopping the tapping, respectively. In the 'Self' condition, subjects were instructed to change behaviors at their will within a block. In the 'Cue' condition, they were forced to follow the rule of green and red crosses. The behavior change in each block was interpreted as an event of a regressor in general liner model (GLM). The GLM was calculated and analyzed using a fixed effect model by SPM8 on Matlab. Results: The inferior parietal lobe (IPL) was commonly activated at behavioral changes except for the passive condition of stopping movement. The middle frontal cortex (MFC) and supplementary motor area (SMA) were activated during a thinking period in the voluntarily conditions compared to the passively cued conditions. Discussion: The current results indicated that brain activations during thinking and motor execution are inseparable. The IPL activation is implicated when the selected behavior (tapping) should be maintained during thinking as it is. The MFC and SMA may subserve active thinking for behavior changes.

Disclosures: **K. Omata:** None. **S. Ito:** None. **Y. Ouchi:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: RIKEN Junior Research Associate Program

Toyota Motor Corporation Grant

Title: Functionally-specific coupling between delta-phase and alpha-amplitude with respect to a choice of a hand

Authors: *T. KAJIHARA[1,2], M. ANWAR[3], M. KAWASAKI[3,4], Y. MIZUNO[3,5,6], K. NAKAZAWA[2], K. KITAJO[3,5]
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Abstract: The motor cortex is known to be the last region to process information before the brain sends the command to the periphery nervous system via the spinal cord. Before the processing in the motor region, there must be a transient formation of neural assemblies to effectively communicate across distributed cortical areas, given any sort of action or cognition requires the integration of information across multiple regions. Furthermore, communication between local oscillators at a global scale in the brain takes longer time and thus would favour slower rhythms with longer temporal window. On top of that, such slower-global oscillations and faster-local oscillations would be coupled to integrate information. Aiming to show such neural dynamics regarding the motor system, we instructed participants to prepare a hand response of the right or left with respect to a non-directional visual stimulus whilst recording their electroencephalography (EEG) activities. As previously known, such covert preparation of a hand response showed functionally specific modulation in alpha (8-14Hz) amplitude; the contralateral motor cortex exhibits relative decrease of alpha amplitude in comparison to the ipsilateral cortex. Prior to this modulation, a number of inter-areal phase synchrony at slower frequencies (2-4Hz) was observed, and especially in the motor regions (C3 and C4) 2Hz phase synchrony was prominent, suggesting that 2Hz phase information evoked alpha-amplitude modulation. To further explore such a relationship, cross-frequency coupling was investigated. Significant delta-phase (2Hz) and high alpha-amplitude (11-14Hz) coupling exhibited functionally-specific modulation; the ipsilateral motor cortex exhibited relative increase of the coupling and the contralateral motor cortex exhibited relative decrease of the coupling. Taken together, the local control of alpha rhythm could be initiated by inter-areal delta phase synchrony.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant #5T32HD055180-03

Title: Enhanced neurobehavioral outcomes of action observation prosthesis training

Authors: *W. CUSACK[1], S. THACH[2], R. PATTERSON[2], D. ACKER[2], R. KISTENBERG[2], L. WHEATON[2]
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Abstract: Proof of principle studies have demonstrated improved neural and behavioral outcomes when prostheses users learn task-specific behaviors by imitating movements of prosthesis users (matched limb) compared to intact limbs (mismatched limb). The current study investigates the cortical activity and corresponding motor behavior in prosthesis users trained with either matched limb or mismatched limb imitation. Intact subjects donned a specially adapted prosthetic device to simulate the wrist and forearm movement that transradial amputees experience. The hypothesis is that non-amputated prosthesis users (NAPUs) trained with matched limb imitation would show greater engagement of the parietofrontal regions and reduced movement variability compared to their counterparts trained with mismatched limb imitation. Training elapsed over three days that were comprised of alternating periods of video demonstration observation followed by action imitation. At the beginning and end of the training protocol, all participants performed a cued movement paradigm while electroencephalography and electrogoniometry were collected in order to track changes in cortical activity and movement variability, respectively. Matched limb participants showed greater engagement of motor-related areas while mismatched limb participants showed greater engagement of the parietooccipital system. Matched limb participants also showed lower movement variability. These results indicate that the type of limb demonstration imitated plays an important role in the neurobehavioral process of learning to use a novel prosthetic device. This finding is important, as customary prosthetic rehabilitation with intact therapists involves mismatched limb imitation that may exacerbate challenges in adapting to new motor patterns demanded by prosthesis use.

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Poster

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Title: Cortical mechanisms for conversion of allocentric target representations into egocentric reach plans in the human

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Abstract: It has been shown that allocentric cues can be used to encode target location in visuo-spatial memory (Obhi & Goodale, Exp Brain Res. 2005; Krigolson et al., Spat Vis. 2007). In our recent fMRI study we have found that occipital and temporal cortices are involved in the coding of allocentric direction (i.e., target location relative to an allocentric landmark) of a remembered reach target, but the egocentric direction coding (i.e., target location relative to gaze) appears in parieto-frontal cortex as soon as participants know the direction of the reach movement (Chen et al., SFN Abst. 2013). However, neural substrates for the allocentric-to-egocentric conversion have not been explored yet. Here we used an event-related fMRI design to investigate brain areas involved in allocentric-to-egocentric conversion of remembered reach targets. Twelve participants reached towards a remembered target location represented in allocentric frames of reference. Participants fixated a central point while a target was presented along with an allocentric landmark for 2s. The concurrent presentation of the target and the landmark was followed by a delay phase (6s), after which an auditory instruction ("Same cue" or "Different cue") instructed participants that the landmark would re-appear at the same location (potentially allowing participants to calculate egocentric reach direction immediately), or at a different location (requiring participants to wait for the re-appearance of the landmark to calculate reach direction). A second delay phase (10s) followed the auditory instruction. Next, the allocentric landmark re-appeared for 2s (at the same or different site as expected) and was followed by a go-signal for reaching towards the remembered target location relative to the re-displayed allocentric landmark. Based on the results of our previous psychophysical experiment (Chen et al., Neuropsychologia 2011), we hypothesized that subjects would convert allocentric information into egocentric information at the first opportunity, and thus the cortical site of this conversion process should be revealed by higher activation in the "Same cue" as compared to the "Different cue" condition during the early portion of the second delay phase. Significant differences in activation consistent with this pattern were observed in bilateral dorsal precuneus, left angular gyrus and bilateral inferior frontal gyrus. These results suggest that specific areas of

posterior parietal and frontal cortex play a critical role in converting allocentric representations of remembered target direction into egocentric plans for reach.

Disclosures: Y. Chen: None. **S. Monaco:** None. **J.D. Crawford:** None.

Poster

443. Cortical Planning of Actions

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 443.18/LL2

Topic: D.17. Voluntary Movements

Support: NIH Grant T32 AS047752

NIH Grant R01 NS059909

NCRR Grant UL1 TR000153

Title: Relationship between M1-M1 coupling during paretic hand movement and corpus callosum integrity after stroke

Authors: *J. C. STEWART[1,2], P. DEWANJEE[2], E. B. QUINLAN[2], L. DODAKIAN[2], A. MCKENZIE[3], J. SEE[2], S. C. CRAMER[2]
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Abstract: Changes in interhemispheric connectivity between the two primary motor cortices (M1) are seen after stroke and can negatively impact motor function in the affected hand. The basis for these connectivity changes is not well understood, but may relate to the structural integrity of commissural tracts. The purpose of this study was to examine the relationship between M1-M1 coupling during paretic hand movement and structural integrity of the motor section of the corpus callosum (CC). Eighteen, right-hand dominant individuals with hemispheric stroke (UEFM 34.3±12.3; days post-stroke 154.6±101.8; 9 left brain damage (LBD), 9 right brain damage (RBD)) completed three functional MRI runs of paretic hand grasp/release. Coupling parameters between the ipsilesional (IL) M1 and contralesional (CL) M1 were extracted from a set of connectivity models using Bayesian model averaging in DCM10/SPM8. Diffusion tensor images (32 directions, 2 mm slices) were used to quantify the structural integrity of the CC motor section in FSL; mean values for fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) were extracted.

Coupling from CL M1 onto IL M1 ranged from +0.52 (facilitatory) to -0.70 (inhibitory) and did not correlate with degree of motor impairment. Across all subjects, M1-M1 coupling did not significantly correlate with FA, MD, RD, or AD in the CC motor section but did show a positive correlation with RD in the IL corticospinal tract (ρ=.503; p=.034); higher RD values corresponded to positive coupling from CL M1 onto IL M1 while lower RD values corresponded to negative coupling. In the LBD group, the magnitude of the coupling from CL M1 onto IL M1 correlated with MD (ρ=.810, p=.015) and RD (ρ=.838, p=.009) of the CC motor section. In the RBD group, M1-M1 coupling did not significantly correlate with any measure of CC structural integrity. The direction of CL to IL M1 connectivity differed based on degree of damage to the IL corticospinal tract; higher RD values, thought to represent greater damage to the myelin sheath, corresponded to facilitation of IL M1 by CL M1 while lower RD values corresponded to inhibition. This finding suggests that access to the IL motor output system may influence the role of CL M1 in paretic hand movement after stroke. Magnitude of M1-M1 coupling correlated with structural integrity of the CC motor section after LBD but not RBD even though level of motor impairment and structural integrity of the CC did not differ between the two groups. Differences in interhemispheric function-structure relationships based on side of brain damage may influence motor recovery and response to motor interventions after stroke.

Disclosures: **J.C. Stewart:** None. **P. Dewanjee:** None. **E.B. Quinlan:** None. **L. Dodakian:** None. **A. McKenzie:** None. **J. See:** None. **S.C. Cramer:** None.

Poster

443. Cortical Planning of Actions

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Program#/Poster#: 443.19/LL3

Topic: D.17. Voluntary Movements

Support: NIH Grant T32 HD057845

NIH Grant R01 NS 058667

Title: Separating descending corticofugal projections in healthy adults using Diffusion Tensor Tractography

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Chicago, IL; [2]NU Interdepartmental Neurosci., [3]Biomed. Engin., Northwestern Univ., Chicago, IL

Abstract: Descending corticofugal projections are a crucial component in human motor control. Although the corticospinal tract has been relatively well described, other components of this important descending projection tract, such as the corticoreticular projection and the corticopontine projections, have not been well characterized in humans. Here we develop a protocol that allows for separation of some individual components of the corticofugal projection in order to separately evaluate their integrity and volume in healthy controls. Healthy adults underwent diffusion tensor imaging on a 3T Siemens TIM trio scanner using an echo-planar based diffusion imaging sequence with diffusion weighting of 1000 s/mm2 in 60 diffusion directions. Images were processed, visualized, and analyzed using the FMRIB Software Library (FSL). Probabilistic tractography was run using the FDT toolbox using masks that defined brainstem regions, posterior limb of the internal capsule and sensorimotor cortical regions. Fractional anisotropy, mean diffusivity and volume were calculated in each tract in both hemispheres. Preliminary data demonstrate reliable separation of corticospinal tract from extrapyramidal projections in all subjects. Additionally, there were no significant differences in volume, fractional anisotropy and mean diffusivity between left and right hemispheres, demonstrating relative symmetry in these projection fibers. This is an important first step towards creating a baseline for later evaluation of tract damage following stroke.

Disclosures: M. Owen: None. **J.P.A. Dewald:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 444.01/LL4

Topic: D.18. Brain-Machine Interface

Support: NIH Grant NS-055236 to RSW

Title: Telemetry-controlled simultaneous stimulation-and-recording device to train cortical circuits in rat somatosensory cortex

Authors: J. T. RAMSHUR[1], A. L. DE JONGH CURRY[1], *R. S. WATERS[2]
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Abstract: Introduction: We previously reported that chronic microstimulation delivered to rat somatosensory cortex (SI) enhances interhemispheric connectivity between homotopic sites and leads to functional changes whereby new responses from the ipsilateral forelimb appeared in ipsilateral SI. Here, we describe the design, implementation, and testing of a telemetry-controlled simultaneous stimulation and recording device (SRD) to deliver chronic microstimulation to physiologically identified sites in rat SI and test hypotheses that chronic microstimulation strengthens interhemispheric pathways and leads to functional reorganization. Design: The SRD is a telemetry-controlled embedded system small enough to be worn by rodents and capable of simultaneous recording and stimulation. The core of the SRD is a Cypress Semiconductor Programmable System on a Chip (PSoC 3) that controls data acquisition, stimulation, and Bluetooth communication. Biopotential signals are amplified and digitized using an Intan Technologies RHD2000 series digital electrophysiology interface chip. The SRD can simultaneously record (1-15 ksps/channel) from any 2 of 12 channels. The SRD can deliver monophasic, biphasic, or pseudo-phasic constant current stimulation waveforms (0-255 uA). Stimulation waveform amplitude and timing parameters are adjustable to the user. The SRD can run autonomously, display continuous data from one channel, or synchronously trigger recording or stimulation events from external devices using infrared pulses. A graphical interface allows users to wirelessly configure SRD experimental parameters, visualize captured waveforms, and store waveforms to data files. Furthermore, the SRD uses commercially available off-the-shelf components and freely available software and source code. Results - bench testing: The SRD operated more than 24 hours using a 1000 mAh battery, produced accurate biphasic current waveforms in saline and *in vivo* using platinum/iridium electrodes, accurately acquired simulated waveforms, and provided a sufficient and reliable wireless connection. Results - *in vivo* testing: The SRD successfully delivered chronic miocrostimulation to physiological identified sites in SI and recorded evoked responses in homotopic sites in contralateral SI. Chronic microstimulation enhanced responses in contralateral homotopic SI that led to the functional expression of new input. Conclusion: The SRD was shown capable of modifying cortical circuits and may have potential applications in stroke rehabilitation and modifying cortical reorganization that stems from phantom limb pain.

Disclosures: J.T. Ramshur: None. **A.L. de Jongh Curry:** None. **R.S. Waters:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 444.02/LL5

Topic: D.18. Brain-Machine Interface

Support: NIMH DP1MH099903

Title: Wheelchair navigation with wirelessly recorded cortical ensembles

Authors: ***P.-H. TSENG**[1,2], S. RAJANGAM[1,2], A. YIN[3,2], G. LEHEW[1,2], D. SCHWARZ[1,2], M. LEBEDEV[1,2], M. A. L. NICOLELIS[1,2,3,4,5]
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Abstract: Controlling a wheelchair through a brain-machine-interface is a significant step as a neuroprosthetic application. Up to date, only noninvasive recordings have been used for wheelchair control, but these suffer from limited bandwidth. Here we show for the first time that invasive, wireless recordings from large ensembles of cortical neurons can enable highly accurate and versatile navigation of a wheelchair. Two rhesus monkeys were chronically implanted with multielectrode recording arrays in multiple cortical areas. We used our recently developed wireless recording system to sample from hundreds of cortical neurons simultaneously. We challenged the monkeys to drive their chairs, mounted on the base of a human motorized wheelchair, by using their cortical ensembles from the primary motor cortex, the supplemental motor area, and the primary somatosensory cortex. Multiple Wiener filters decoded the monkeys' cortical activity into the navigational signals: forward or backward velocity and turns. Both monkeys successfully acquired the ability to independently steer the wheel chair towards a grape reward in an open area using their cortical activity. They learned to achieve this task with multiple car starting positions and orientations. The monkeys learned the task within a short span of time and developed efficient control of the car. This learning was accompanied by adaptive changes in cortical modulations to the wheelchair movements. We suggest that such neuronal adaptations underlie an incorporation of the wheelchair in cortical representation of the body schema.

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Poster

444. Brain–Machine Interface: Implanted Electrodes I

Location: Halls A-C

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Program#/Poster#: 444.03/LL6

Topic: D.18. Brain-Machine Interface

Support: NSFC Funding U1304602

Title: Correlation analysis of local field potentials in pigeon's nidopallium caudolaterale to decoding its steering motion

Authors: *Z. SHANG[1], X. LIU[2], Z. LI[2], Z. WANG[2], H. WAN[2]
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Abstract: Abstract: We investigate whether the neural activity of in the pigeon's nidopallium caudolaterale (NCL) involves encoding of motion. The 16 channels LFPs in NCL of pigeon are recorded when it turned left or right in customized L-shape maze respectively. We use the sparse decomposition method to learn the dictionaries corresponding to the different steering motion by training of temporal correlation metric of inter-channel LFPs. We found there is the obvious difference between the correlation patterns of left and right steering in some specific time course. The result of decoding experiment indicates the neural population information extracted from LFPs in pigeon's NCL could be used to identify its intention of steering motion. Introduction: the NCL of the endbrain in pigeons has been identified as a key cognitive brain region, similar to the PFC in mammals. Now it is positioned to integrate sensory input and project to motor output. Thinking the neural activity of in the pigeon's NCL maybe involves encoding of motion, so we investigate whether the LFPs information in NCL of pigeon could reflect some motion intension. Methods: One 1-year old male pigeon is selected as subject, with the 16 channel microelectrode arrays implanted chronically in its NCL region. We trained it to turned left or right in customized L-shape maze. 16 channels LFPs are recorded in some time including steering motion happen. We use the sparse decomposition method to learn the dictionaries corresponding to the different steering motion by training of temporal correlation metric of inter-channel LFPs, which are sampled randomly in 50ms. Results: there is the obvious difference between the correlation pattern of left steering and ones of right steering in some specific time course($p<0.05$). Figure 1 is some examples of correlation pattern of inter-channel LFPs corresponding to left and right. In decoding experiment, We use the sparse representation reconstructing errors as features and the support vector machine as classifier. The classification correct rate is above 90%.

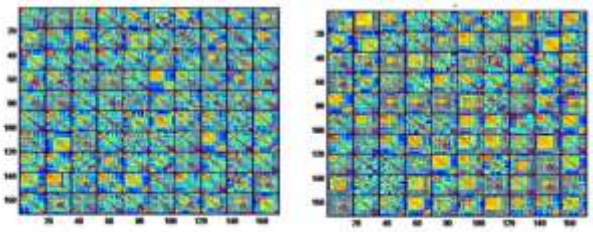

Figure 1 examples of correlation pattern of inter-channel LFPs

Disclosures: **Z. Shang:** None. **X. Liu:** None. **Z. Li:** None. **Z. Wang:** None. **H. Wan:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Program#/Poster#: 444.04/LL7

Topic: D.18. Brain-Machine Interface

Support: R01EY013337

Title: Perturbation of state-estimation in area 5d

Authors: ***L. S. URBAN**, Y. SHI, M. HAUSCHILD, R. ANDERSEN
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Abstract: Neural activity in area 5d of the posterior parietal cortex (PPC) is known to be involved in reaching movements. Historically, PPC has been thought to contain high-level cognitive signals, such as goals and intentions. Recent studies have shown that area 5d also encodes a signal correlated to real-time hand kinematics. Since this brain area has direct applications in the field of neural-prosthetics, it is important to understand the underlying nature of this signal. One effective approach is to analyze the temporal offset between neural activity and the real-world kinematics. In the case of a command signal, neural activity must precede movement, to account for the synaptic delays driving muscle activity. In the case of a sensory signal, neural activity must follow movement, to account for synaptic delays of the incoming signal from the perceptual system. Previous studies have shown that the offset timing in area 5d is both too slow to encode a command signal, and too fast to encode a sensory signal (Mulliken et al., 2008). We believe that area 5d contains an internal estimate of kinematics, which is updated via perceptual feedback. To explore this idea, two rhesus macaque monkeys were implanted with chronic multi-electrode arrays (FMA/Utah) in area 5d. One animal was implanted with a second array (Utah) in motor cortex (M1) to serve as a control. The animals performed reaches using a virtual reality setup in which an artificial visual lag between hand and cursor position was introduced. We found that, immediately after the onset of the lag, there is a significant drop in signal fidelity in area 5d. As the session progresses the signal strength returns to its original level, but with no significant shift in offset timing. Additionally, the population encoding appears to adjust during the lag condition, such that the signal is embedded in a different format than during the initial no-lag condition. During washout, the signal quickly readapts, and the population encoding returns to the original format. No noticeable effect was

seen in M1. These results suggest that area 5d does not encode a purely sensory signal. Instead, it contains an adaptive internal estimate of the current state of the hand, which compensates for this visual lag. This signal type would be ideal for neural prosthetics, because it encodes the intended position and not the result of the action plan, which may be erroneously executed.

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Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: Defense Advanced Research Projects Agency, Microsystems Technology Office (DARPA, MTO) Reliable Neural Interface Program through an Interagency Agreement with the FDA (FDA-DARPA 224-10-6006).

Title: Spectral fingerprinting: Reproducible unsupervised assessment of spiking activity in multisite electrode recordings

Authors: *E. F. CIVILLICO, C. G. WELLE, K. RUDA, K. WACHRATHIT, T. HEARN, V. KRAUTHAMER
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Abstract: Variability in the long-term performance of implanted penetrating electrode arrays is an important concern for therapeutic and research applications. Published experimental uses of multisite arrays include neuroprosthetics systems, which provide mechanical or virtual assistance for paralyzed or amputee patients through a direct interface with the nervous system, as well as therapeutic uses to treat epilepsy and Parkinson's disease. As the number of recording sites and the resolution of the captured data scales beyond what can reasonably be assessed by trained experts in real time, it will become increasingly important to find automated and objective methods for assessing neural data and extracting meaningful features. Here we propose a cohesive set of visualization, artifact rejection, quality assessment, and feature extraction strategies designed to optimize the use of multi-unit extracellular data for therapeutic and research purposes. In the first stage, high-pass filtered channels with severe artifacts are identified by assessment of their spectral content and eliminated from further analysis. In the second stage, less severe artifacts are removed from the remaining channels by common average

referencing. Finally, the spiking content of each channel is scored as the power in a 1 kHz-wide frequency band centered on 1 kHz. By quantifying the number of channels eliminated in the first stage, the properties of the common signal removed in the second stage, and the spiking power in the final stage, we obtain an assessment of the neural interface that relies on minimal assumptions and is directly comparable across widely disparate preparations, and over long implant times within the same preparation. We report on the application of this method to 190 hours of data recorded in 15 minute sessions over many months from 21 awake behaving mice, each implanted either with a single-shank Neuronexus Technologies A16 probe, a Tucker-Davis Technologies 2x8 tungsten microwire array, or a Blackrock Microsystems 4x4 floating platinum-iridium array. The results of our rapid automated assessment are compared with results obtained from manual cluster-cutting. We suggest that the use of recording evaluation metrics corresponding to objective physical quantities is advantageous for reproducible benchmarking of emerging technologies. Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

Disclosures: E.F. Civillico: None. **C.G. Welle:** None. **K. Ruda:** None. **K. Wachrathit:** None. **T. Hearn:** None. **V. Krauthamer:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: DARPA REPAIR N66001-10-C-2010

NIH NEI EY015679

PVA 2978

Title: Mitigating electrical stimulation artifacts for bidirectional neural interfaces

Authors: *J. E. O'DOHERTY, P. N. SABES
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Abstract: Electrical stimulation of the nervous system is a successful tool for addressing basic neuroscience questions and for providing effective clinical therapies. Stimulation has been used

to causally perturb neural circuits, treat movement disorders and restore sensory function. Recently there has been interest in the creation of bidirectional interfaces with the brain, for example, brain-machine interfaces (BMIs) augmented with somatosensory feedback. A major challenge for such bidirectional interfaces is the interference caused by electrical stimulation artifacts on concurrent electrical recordings. Stimulation artifacts obscure ongoing neural activity (false negatives), can be misinterpreted as neural activity (false positives), and can distort the shape of extracellular potentials, affecting action potential discrimination. Moreover, stimulation artifacts may be variable across multiple repetitions of stimulation pulse trains, or even from pulse to pulse. Neural interfaces that employ multiple independent channels of stimulation pose a particular challenge for concurrent recordings as intervals uncontaminated by stimulation can be severely limited and because the stimulation artifacts interact. The ideal stimulation artifact removal method would decrease the magnitude of artifacts, their temporal extent and their variability. Additionally, we are particularly interested in artifact mitigation strategies that can be implemented on-line and in real-time, thereby enabling closed-loop operations. Here we evaluate strategies for reducing the impact of electrical stimulation through modifications in stimulation protocol and online filtering of the extracellular potential. These approaches were validated *in vivo* in a rhesus monkey model using Utah electrode arrays implanted in sensorimotor cortex and Tucker-Davis Technologies stimulator and amplifier. Intracortical microstimulation (ICMS) was provided to channels in primary somatosensory cortex (S1) while recordings were made in primary motor cortex (M1). Specifically, we investigated: 1) bipolar versus monopolar stimulation, 2) the impact of bandpass filtering on artifact shape, 3) temporal median filtering 4) template subtraction and 5) adaptive linear least squares filtering. These strategies will be applied to enable next generation bidirectional neural interfaces.

Disclosures: **J.E. O'Doherty:** None. **P.N. Sabes:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: National Science Foundation grant EFRI-M3C 1137267

Defense Advanced Research Projects Agency contract N66001-10-C-2008

Title: Neural control strategies in a closed-loop brain-machine interface with a 4 degree of freedom redundant actuator

Authors: *H. G. MOORMAN[1], S. GOWDA[2], J. M. CARMENA[1,2]
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Abstract: The development of skilled control of brain-machine interface (BMI) systems has been shown for a variety of species, decoding algorithms, and movement actuators. Typically, the number of kinematic parameters that are directly controlled by neural activity in such systems is equal to the number of degrees of freedom of the actuator, such that there is a single kinematic solution for any desired endpoint movement. During natural motor control of the arm, however, there are often multiple possible kinematic solutions resulting in a particular endpoint movement (e.g. many different configurations of hand, arm and shoulder joints can result in the tip of the finger moving along a single path in space). To study how subjects control a BMI exhibiting this type of redundancy, we designed a virtual four-link arm with the movement of the joints confined to a single 2-D plane. This paradigm enables kinematic redundancy in the BMI movements, as there are two more degrees of freedom than necessary to perform a planar reaching task. We recorded single- and multi-unit neural activity from chronic electrode arrays implanted in the primary motor cortex of a rhesus macaque monkey and transformed the recorded activity into the angular positions and velocities of each of the four joints using a Kalman filter. Using this system, the subject performed an on-screen target hitting task with the endpoint of the four-link arm. To test whether the subject could successfully map multiple kinematic solutions to the same intended endpoint movement, we varied the initial configuration of the arm joints for a given endpoint location at the beginning of each trial. We found that the subject was able to adapt the joint movement in order to carry out the desired endpoint movement to the target regardless of the starting configuration. This result indicates that subjects are able to select from among multiple redundant kinematic solutions when the task demands it. Additionally, this experimental paradigm may provide a useful framework for future studies of neural strategies for selecting among motor-equivalent solutions.

Disclosures: H.G. Moorman: None. **S. Gowda:** None. **J.M. Carmena:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Program#/Poster#: 444.08/LL11

Topic: D.18. Brain-Machine Interface

Support: NIH (NIMH) Grant DP1MH099903

Title: Computing arm movements with a monkey brainet

Authors: *P. IFFT[1,2], A. RAMAKRISHNAN[2,3], M. PAIS-VIEIRA[2,3], Y. BYUN[1,2], K. Z. ZHUANG[1,2], M. A. LEBEDEV[2,3], M. A. L. NICOLELIS[1,2,3,4,5]
[1]Dept. of Biomed. Engin., [2]Ctr. for Neuroengineering, [3]Neurobio., [4]Psychology and Neurosci., Duke Univ., Durham, NC; [5]Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal, Natal, Brazil

Abstract: Many organisms exhibit cooperative behaviors through which several individuals coordinate their actions to achieve a common goal. Previously, brain-machine interfaces (BMIs) have extracted motor commands from a single brain to communicate with external devices. Here, we developed the first shared BMI that engages groups of animals in a common motor behavior using simultaneously recorded very-large-scale brain activity from all subjects. This new paradigm, termed a Brainet, was comprised of two (B2) or three (B3) rhesus monkey brains working together in three different configurations: (1) a B2 driving 2D movements of an avatar arm where each monkey had 50% control of both X and Y axes, (2) a B2 generating 2D arm movements where one brain fully controlled the X axis and the other was in full control of the Y axis, and (3) a B3 producing movements of a single avatar arm in 3D space, while each individual monkey viewed and generated movements in 2D subspaces (X-Y, Y-Z, or X-Z respectively). Long-term cooperation via a Brainet induced functional modifications in each primate's brain and led to the emergence of collective behaviors. These included increased synchronization between brains, concurrent plastic adaptations to task contingencies, and cooperative behaviors that minimized individual effort and enabled conjoint action. These results suggest that primate brains can be integrated into a self-adapting hybrid computing engine, capable of achieving a common motor goal.

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Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Program#/Poster#: 444.09/LL12

Topic: D.18. Brain-Machine Interface

Title: Limits on transmission of information in primary motor cortex during multidimensional reaches

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Abstract: Brain-computer interfaces (BCIs) show promise for restoring interaction with the external world for individuals that have lost that ability through spinal cord injuries, strokes or amputations. As work has progressed in BCIs, there has been a continuously increasing number of degrees of freedom (DoF) controlled in the tasks. BCI experiments have progressed from two DoF control of a computer cursor to seven DoF control of a robotic arm. Current research seeks to control a robotic arm with nine DoFs within the hand and six DoFs of translation and orientation. Faced with this demand for increasing control, it becomes important to understand the limits of the neural population controlling the system. The neural populations and decoding schemes used in BCI work can be considered as a throughput channel. The desired output state passes through this channel, with some noise, to become the actual output state. In the context of information theory, such systems have limits on information transmission due to the noise. As the task constraints and controlled DoFs increase, the information demand on the population also increases. Thus, the questions remain as to whether and how the neural population accommodates to this increasing information demand to allow for stable, reliable control. To answer this question, we recorded from populations of neurons in primary motor cortex (M1) while a monkey performed center-out reaches in two and three-dimensional tasks. We developed an algorithm using factor-analysis dimensionality reduction to estimate the mutual information between the neural response and the set of targets. In both the two and three-dimensional contexts, the target entropy was varied by increasing the target number while decreasing the target size. This was done in order to observe how the mutual information estimate changes as the target entropy increases. As predicted, the mutual information estimate increases up to an asymptotic level with increasing target entropy, reaching a limit of information transmission for the system. Furthermore, for identical neural populations and the same number of targets, the mutual information level is lower in the two-dimensional task compared to the three-dimensional task. This suggests that the neural population increases its information content when an additional dimension is added to the task. Further work will study the effects of higher dimensional tasks and population size as well as whether the information limits are maintained in BCI tasks.

Disclosures: **R.G. Rasmussen:** None. **S.M. Chase:** None. **A.B. Schwartz:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 444.10/LL13

Topic: D.18. Brain-Machine Interface

Title: Joint inference for spike sorting and decoding for brain-machine interface

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Abstract: Brain-machine interfaces (BMIs) are devices that record and decode neural activity to control assistive devices. Among the most important elements of BMI data processing software are the spike sorting and decoding algorithms. Classical spike sorting algorithms have revolved around grouping observed spikes into well separated clusters but have rarely utilized the properties of neuronal task-related modulations. For the purposes of a BMI, however, task-related activity and decoding performance matters much more than whether spikes were grouped into distinct clusters. This suggests that spike sorting and decoding algorithms should inform each other about how to group waveforms so that decoding error is minimized. This work presents an algorithm that jointly learns sorting and decoding parameters. The algorithm makes use of the stick-breaking construction of the Dirichlet process Gaussian mixture model to learn the number of neurons that surround an electrode and the parameters that characterize them and employs a standard Kalman filter to decode kinematics. It is fully Bayesian; model parameters and their uncertainties can be quantified through Gibbs sampling. A BMI performance metric, that includes correlation with the true kinematic trace and the mean squared error between the predicted and true kinematic traces, is employed to quantify performance. Results on simulated and experimental data show that the algorithm significantly improves decoding performance when the spread of clusters is very tight. Furthermore, the algorithm is fast enough for real-time spike sorting and decoding, making it practical for online BMIs.

Disclosures: **V.A. Subramanian:** None. **D. Carlson:** None. **M.A.L. Nicolelis:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Program#/Poster#: 444.11/LL14

Topic: D.18. Brain-Machine Interface

Support: NDSEG

Title: Goal-directed modulation of neural activity in rodent primary visual cortex via a brain-machine interface

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Abstract: Top-down modulation of visual processing is a fundamental component of cognitive tasks like selective attention and visual working memory. Facilitation of task-relevant representations and suppression of distracting representations has been observed in the visual system as early as the primary visual cortex (V1). To characterize the circuits involved in top-down modulation in the visual cortex, we trained freely behaving rats to perform a brain-machine interface (BMI) task using control signals recorded in V1. Well-isolated single units were recorded from animals chronically implanted with microwire arrays in the deep layers of V1. Units were arbitrarily assigned to one of two ensembles, and the difference of binned spike activity between both ensembles was mapped to the pitch of an auditory cursor. Animals learned to modulate the tone pitch to hit a high frequency target that was linked to liquid reward. The number of correct trials rose above chance rate after only a few days of training, and performance increased across days as well as within sessions. BMI task performance was sensitive to reward contingency, suggesting that performance was goal-directed rather than habitual. Additionally, manipulating ambient light conditions interfered with animals' ability to perform the task, but only temporarily. Increases in coherence in the beta and gamma bands were observed between task-relevant neurons and local field potentials (LFPs) around the time of target achievement, but not between task irrelevant neurons and LFPs. These results suggest that BMI can be a useful paradigm for studying the cellular and circuit-level dynamics underlying goal-directed modulation in sensory cortex.

Disclosures: R. Neely: None. **A. Koralek:** None. **J. Carmena:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: Donation from Itau Bank

NIH (NIMH) Grant DP1MH099903

National Center of Competences in Research (NCCR) Robotics

Title: Cortical control of a lower-limb exoskeleton in rhesus monkeys

Authors: *K. Z. ZHUANG[1,2], T. VOUGA[5], J. OLIVIER[6], M. BOURI[6], H. BLEULER[6], M. A. LEBEDEV[3,2], M. A. L. NICOLELIS[3,2,1,4,7]
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Abstract: Previously, we have shown that kinematic parameters of lower limb motion can be extracted from cortical ensembles recorded in rhesus monkeys trained to walk bipedally. However, translating these findings into a real-time implementation of a brain-machine interface for locomotion still remains a challenge. Here, we report the first bipedal exoskeleton continuously controlled by ensembles of cortical neurons. The exoskeleton is driven by four brushless DC motors, each controlling one joint of each leg. The structure of the exoskeleton parallels that of the monkey's biomechanics and is adjustable for monkeys of different sizes. Monkeys are restrained in the exoskeleton only by the foot plate, shank cuff and waist restraint. Hundreds of neurons are recorded from multiple cortical areas with chronically implanted multielectrode arrays. A classical tethered or novel wireless recording system sends neural data to a nearby computer, which is responsible for filtering and decoding. The computer then sends the decoded kinematic parameters to the embedded controller of the exoskeleton. The controller is responsible for accurately actuating the motors to the decoded positions. Force exerted by the monkey can be continuously monitored and also predicted. Currently, the monkey receives visual feedback about the exoskeleton's position on a computer monitor (cursor position or a realistic whole body avatar) as well as sensory feedback from the movements of the exoskeleton. Monkeys learn to manipulate the exoskeleton kinematics by pursuing a moving screen target with the cursor. Our preliminary experiments have already shown that monkeys can improve in continuous cortical control of the exoskeleton, which suggests that this approach can be used in clinical applications for human patients in the future.

Disclosures: K.Z. Zhuang: None. **T. Vouga:** None. **J. Olivier:** None. **M. Bouri:** None. **H. Bleuler:** None. **M.A. Lebedev:** None. **M.A.L. Nicolelis:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Program#/Poster#: 444.13/LL16

Topic: D.18. Brain-Machine Interface

Support: A*STAR Neurodevice grant

Title: Is spike sorting necessary to achieve maximal decoding accuracy?

Authors: *R. SO[1], Z. XU[1], C. LIBEDINSKY[2], C. GUAN[1]
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Abstract: Spike detection and spike sorting are often the first steps towards decoding brain signals. However, whether spike sorting is necessary remains unclear. One hypothesis is that multiunit activity is sufficient for decoding since neurons located close to an electrode have similar directional tuning and individual units do not provide additional information. We tested this hypothesis using single-unit recordings from a macaque performing tasks requiring hand control of a joystick. METHODS: One male rhesus macaque was trained to perform two tasks using a hand-held joystick - the first was to move a computer cursor into a yellow square; the second was to drive a moving platform towards a target. Three 32-channel (CH) microelectrode arrays were implanted in the primary motor cortex, and wideband as well as single unit activity were recorded while the monkey was performing each task. Thresholding and spike sorting were performed manually before the start of each session. Four categories of joystick movement were allowed - front/up, right, left, and stop, and decoding accuracy was determined offline by comparing the decoded direction during each 100 ms bin to the actual joystick signals. RESULTS: Out of 21 CHs with spike activity, 13 CHs had only one unit, while 8 CHs contained multiple units. Using sorted single unit activity, decoding accuracy was 89.8% (±1.4%) for the cursor task, and 75.2% (±1.8%) for the mobile platform task. After combining activity for channels with multiple units, decoding accuracy significantly decreased for both the cursor task ($p=0.0012$) and mobile platform task ($p=0.0091$). A second spike detection algorithm was applied, and multiunit activity detected in this manner resulted in decoding accuracy that was not significantly different compared to sorted spikes ($p>0.05$). Among the 8 CHs with multiple units, only 4 CHs (50%) contained units with similar directional tuning. Thus, combining all spikes in some multiunit channels resulted in a loss of directional selectivity. Automatic thresholding preserved directional selectivity in most channels since smaller units were disregarded. CONCLUSION: Spike sorting did not significantly improve neural decoding of movement

directions. However, this does not indicate that units within the same channel have similar directional tuning. An inclusion of all possible spikes in a channel may result in a loss of selectivity for movement direction; and only with an appropriate threshold, unsorted spikes were able to achieve similar decoding accuracy. Bypassing spike sorting saves effort and computational time, which is important when designing wireless transmission systems for a brain machine interface.

Disclosures: **R. So:** None. **Z. Xu:** None. **C. Libedinsky:** None. **C. Guan:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

Location: Halls A-C

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Support: American Heart Association

VA CDA

Burroughs Wellcome Fund

Title: Robust neuroprosthetic control from the immediate perilesional cortex

Authors: ***K. GANGULY**, C. WONG, S.-J. WONG, R. A. SWANSON, T. GULATI
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Abstract: Introduction: Stroke is a leading cause of motor disability worldwide. There is great interest in the development of methods to both enhance recovery and to allow control of assistive devices in those who do not recover. Brain-Machine Interfaces (BMI) that monitor neural activity from the injured perilesional cortex have the potential to do both. Fundamental questions for the development of BMIs tailored to the perilesional cortex are: (1) What are the electrophysiological characteristics of the perilesional cortex; and (2) Can perilesional networks be volitionally controlled in a manner similar to the intact motor cortex? Methods: We tested the capacity for direct volitional control of neurons in five Long-Evans rats that had 16-32 channel microwire arrays implanted in the rostral perilesional cortex. The upper-limb primary motor stroke was induced using a photothrombotic method. We also implanted five healthy rats as a control group. We compared the ability of each group to match neural activations to simple (i.e. either increases or decreases in firing rate) and more complex transforms (i.e. learning random

weights requiring simultaneous increases and decreases) to control the rotation of a feeding tube (i.e. neuroprosthetic control). The effectiveness of the stroke was assessed through deficits in a reach-to-grasp task. We assessed the neural yield and neuroprosthetic performance of the units in the 'near', 'mid' and 'far' regions from the stroke (250 μm, 750 μm & 1500 μm from the edge of the stroke). This was confirmed through electrolytic lesions & micro-CT. Results: In the initial post-stroke period, we found a spatiotemporal evolution of the recorded neural signals on the near versus far electrodes (i.e. lowest yield most proximal to the stroke). After approximately 2 weeks, the yields were similar for all channels. We found that all regions in the perilesional cortex were as effective as the healthy motor cortex in their ability to control the feeding tube using either transform. Moreover, this was possible from the very outset after the appearance of spiking activity. We also did not find any differences (i.e. time to task completion, slope of learning) between the injured and the healthy motor cortex. The depth of modulation, i.e. the changes in firing rate before & after learning, was also comparable between the two groups. Conclusion: We found the perilesional cortex to be a robust target for a BMI. Surprisingly, we did not find significant differences from a healthy motor cortex. Our results suggest that the perilesional cortex could be a viable target for BMIs specifically designed for patients with long-term deficits after stroke.

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Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: KAKENHI 18020030

KAKENHI 18047027

JST PRESTO

JSPS SP11034

Title: Posture dependency of the twitch responses induced by intraspinal microstimulation to the primate spinal cord

Authors: H. YAGUCHI[1], D. KOWALSKI[2], T. TAKEI[1], *K. SEKI[1]
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Abstract: Proper execution of voluntary movement requires the sensorimotor transformation based on the initial state of limbs. For example, successful reaching to a stable target requires the recruitment of different group of muscles depends on the limb position upon the movement initiation. To test if this transformation could be occurred as early as the spinal level, we stimulated cervical spinal cord of anesthetized monkeys at different arm postures and examined the modulation of twitch response induced in the upper limb muscles. Each monkey laid prone on a table and the left forearm was fixed at the wrist on one of the 7 points on a square grid (8 cm interval) with a multi-axis force sensor during stimulation. A multichannel microelectrode array (Floating Microelectrode Array: FMA) was implanted into C6 segment of the spinal cord and electromyographic (EMG) electrodes were implanted to 12 limb muscles (5 hand, 4 elbow, and 3 shoulder muscles). Magnitude and onset latency of evoked response in each electrode-muscle pair has examined by changing hand position systematically at 9 positions in a horizontal plane with the monkey prone position. Threshold current for each FMA electrode were ranged within 50-670 μA. In three monkeys, we examined the ISMS-induced response for a total of 330 electrode-muscle pairs. Among them, 61% of pairs exhibited significant modulation of either magnitude or latency of twitch responses by changing hand position (posture-dependency). We found the posture-dependency of the magnitude and latency of evoked response preferentially occurred in the distal than proximal muscle, but not affected by the location of electrode within the spinal segment where we stimulated. This posture dependency could be induced by ensemble activity of peripheral afferent, either via direct pathway (e.g. segmental reflex pathway) or more indirect pathway through brainstem and cortex. To dissociate these direct and indirect mechanisms, spinal cord were transected at upper cervical level (C2) and compared the posture-dependency before and after the transection. We found that the posture-dependency did not affected by the spinalization, suggesting that the posture-dependency is largely generated through intra-spinal mechanism via direct pathway. Overall, these results suggest that excitability in the wide area of cervical spinal cord was affected by arm posture through spinal reflex pathways. In the control of voluntary arm movement, this posture-dependency of spinal motor output could have an affect on the descending motor command by calibrating them according to the initial posture of arm.

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Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Title: Studies of electrocortical recordings from a newly conceived chronically-implanted wireless device in monkeys

Authors: *G. E. BIELLA, A. G. ZIPPO
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Abstract: Introduction: Artificial Brain-Machine Interfaces (BMI) represent a prospective step forward the vicarial support or replacement of faulty brain functions. The width and intricacy of replacement of a "brain function" is significantly related to the extent and complexity that the function spans in the neural context. Another issue is the "ecological niche" the interface may best occupy in the surviving neural context and its coherence with the residue functions. Namely, the device must neither interfere nor disorganize the already existing information background in the plan of potential clinical requirement as rehabilitation, adjutancy or functional replacement. A prerequisite from a chronically implantable device is also represented by the ease of reciprocal conveyance of fine-grain information from and to the brain. Not least, a BMI must meet the requisite of long term compliance within a delicate context such as the nervous tissue, without provoking rejection responses. Methods and Results: We present here the electrophysiological results obtained from a non-human primate (Macaca fascicularis) chronically implanted with a novel implantable BMI platform, called Cyberbrain, a 16 channel totally wireless grid, rechargeable by induction [AB Medica (Milan, Italy), designed by one of the authors, PR]. The grid provides both the wireless transmission of epicortical recordings and, equally, the delivery of finely driven stimulations. The grid was implanted (by PR) over the sensorimotor cortex (13 electrodes over the primary motor cortex, 3 on the primary somatosensory cortex) in the deeply anaesthetized animal. Cortical sensory and motor recordings and stimulations have been performed during 6 months. In details, by motor cortex epicortical single spot stimulations (1 to 8V, 1 to 10 Hz, 500us, biphasic waves) we analyzed the motor topographic precision, evidenced by tunable finger movements of the anesthetized animal. The responses to light mechanical peripheral sensory stimuli (50 stimuli, 1ms, variable delays 1.5 to 4 s) were analyzed, both in ongoing spontaneous activity and after activations of each epicortical sensory lead. In the first, we investigated, by estimating the mutual information between the single lead activities, the detection dynamics of the responses to peripheral stimuli within a sensory cortical circuitry, in the second we evaluated the grid electrical interference with somatotopic natural stimuli sensory detection programs. Conclusions: These features provide important technical suggestion for long-term implanted BMI and help for future therapeutic applications in sensorimotor and neurodegenerative diseases.

Disclosures: G.E. Biella: None. **A.G. Zippo:** None.

Poster

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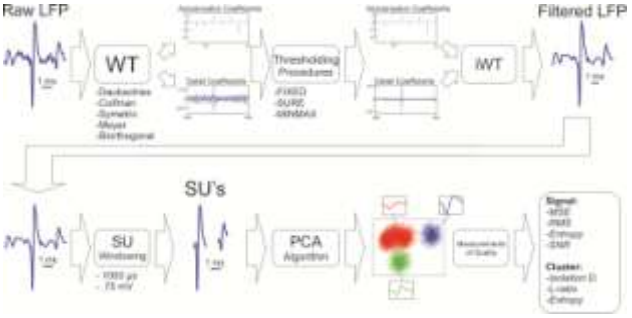
Topic: D.18. Brain-Machine Interface

Support: NIH NINDS R01 NS37822

Title: Wavelet transform preprocessing methodology to improve single unit isolation in primary motor cortex cells from a macaca fascicularis

Authors: *A. ORTIZ-ROSARIO[1,2], H. ADELI[2,3], J. A. BUFORD[1,2,3,4]
[1]Sch. of Hlth. & Rehabil. Sci., [2]Biomed. Engin., [3]Neurosci., [4]Div. of Physical Therapy, The Ohio State Univ., Columbus, OH

Abstract: The proper isolation of neurons is an active area of research in the fields of neuroscience and biomedical signal processing. This project presents a neuron isolation methodology using the wavelet transform (WT), a statistical thresholding scheme, and the principal component analysis (PCA) clustering algorithm on a local field potential (LFP) signal. After signal decomposition via WT, the statistical thresholding schemes select the most adequate coefficients for reconstruction of the LFP. Effectiveness of five different mother wavelets is investigated (biorthogonal, Daubachies 4, discrete Meyer, symmetric 4, and Coifman 4) along with three different wavelet coefficient thresholding schemes (fixed form threshold, Stein's unbiased estimate of risk, and minimax) and two different thresholding rules (soft and hard thresholding). The signal quality is evaluated using four different statistical measures: mean-squared error, root-mean squared, Shannon's entropy, and signal to noise ratio. The clustering quality is evaluated using three different statistical measures: isolation distance, L-ratio, and cluster Entropy. This research shows that the selection of mother wavelet influences in the clustering and isolation of the neuron with symmetric 4 performing the best. This methodology has broad applications in the neuroscience field where neuron isolation is important.



Disclosures: **A. Ortiz-Rosario:** None. **H. Adeli:** None. **J.A. Buford:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: DARPA Grant FDA-DARPA 224-10-6006

Title: Optogenetic investigation of functional neuronal changes proximal to chronically implanted microelectrode arrays

Authors: ***G. L. KNAACK**[1], T. HEARN[2], K. RUDA[2], S. HUANG[2], K. T. WACHRATHIT[3], V. KRAUTHAMER[2], C. G. WELLE[2], E. F. CIVILLICO[2]
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Abstract: Implanted penetrating microelectrode arrays sample neural data at high spatial and temporal resolution, providing a rich source of input for neuroprosthetics and other potential therapeutic interventions. At present, the utility of these implants may be limited by the degradation of recorded signals over time, an effect that may have both biotic and abiotic causes. Biotic sources of signal loss may include changes to neuronal viability resulting from the brain's foreign-body response to electrode implantation. Published reports differ on the histologic effects on neurons near implants, and very little data exists which can distinguish between potential cellular toxicologic effects and circuit remodeling effects. Here we report an *in vivo* optogenetic assay to detect changes in neuronal function near implanted microelectrode arrays. 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. Following one week recovery, weekly recordings were performed from awake mobile mice. Each recording consisted of 10 minutes of spontaneous activity, followed by optical stimulation (473 nm, 10 mW output power measured at the brain surface) with 5 second trains (5, 10, 20, 50, and 100ms pulses at 1, 5, 10 and 40 Hz). Recordings were made for at least 3 months while spontaneous and optically-evoked activities were quantified to assay for the presence of viable neurons. The magnitude of optically-evoked multiunit activity was dependent on stimulation duration and frequency. Optically driven spikes

persisted for 5-10ms after stimulus onset; thus longer pulses did not drive activity for the entire duration of the pulse. Preliminary data showed that the largest responses declined by approximately 50% by 13 weeks post-implant, while smaller responses were more consistent over long time scales. The decline in the largest responses tracked the decline observed in spontaneous spiking, suggesting a gradual functional change rather than an abrupt silencing. Adaptation was observed and found to be dependent on both pulse frequency and postimplant time. The data suggest a combination of neuronal loss and altered circuitry proximal to chronically implanted microelectrode arrays; however, alternative interpretations include abiotic degradation of the recording device or alterations in transgene expression level. Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

Disclosures: **G.L. Knaack:** None. **T. Hearn:** None. **K. Ruda:** None. **S. Huang:** None. **K.T. Wachrathit:** None. **V. Krauthamer:** None. **C.G. Welle:** None. **E.F. Civillico:** None.

Poster

444. Brain–Machine Interface: Implanted Electrodes I

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Topic: D.18. Brain-Machine Interface

Support: Defense Advanced Research Projects Agency, Microsystems Technology Office (DARPA, MTO) Reliable Neural Interface Program through an Interagency Agreement with the FDA (FDA-DARPA 224-10-6006).

Title: Evaluating the long-term effectiveness of neural interface technology with a spontaneous behavior classification platform

Authors: S. HUANG[1], E. F. CIVILLICO[1], G. L. KNAACK[2], T. HEARN[1], V. KRAUTHAMER[1], *C. G. WELLE[1]
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Abstract: Intracortical neuroprosthetic systems hold promise in translating neural activities into command signals for the control of prosthetic devices. However, chronically-implanted intracortical electrodes experience a longitudinal reduction in the ability to detect neural signals

in both human patients and animal models, resulting in a gradual reduction of neuroprosthetic control capabilities. Signal decline is often quantified on the basis of identified single unit number or action potential amplitude, or multiunit threshold crossing rate. While these metrics capture neural activity levels in the cortex, they do not predict the functional relevance of the observed neural signals. The strength of the correlation between neural signal characteristics in the motor cortex and motor output behavior may serve as an additional metric of neural implant reliability. To determine the longitudinal behavioral relevance of neural signals recorded by multi-electrode arrays, we implanted commercially-available electrode arrays (NeuroNexus, Blackrock Microsystems, Tucker-Davis Technologies) in the caudal forelimb area of the motor cortex of the mouse and recorded biweekly over time scales of 1 to 12 months post-implantation. Video recordings of the animal locomotion were simultaneously acquired using a video-based behavioral system (HomeCageScan, CleverSys Inc.) to automatically classify spontaneous rodent behaviors into forelimb and non-forelimb movements. Behavior-locked longitudinal electrophysiological recordings were evaluated for changes in functional relevance. Data acquired with this test platform revealed significant differences between neural activities associated with front-limb and non-front-limb behaviors, and both groups experienced a decline in signal over time. Preliminary data suggests that the ratio of front-limb to non-front-limb associated activity remained constant for implantation duration, implying that functional relevance of multiunit neural activity in the cortex remains stable during electrode implantation. Combined behavioral and electrophysiological monitoring of neural implant devices may potentially be utilized as a test platform to evaluate safety and efficacy issues related to invasive neurological devices. DISCLAIMER The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

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Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

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Topic: D.18. Brain-Machine Interface

Support: DARPA MTO through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-11-1-4120

Title: The spatial distribution and intensity of FBR biomarkers in rat cortex correlates with silicon microelectrode array recording performance

Authors: *N. NOLTA, M. B. CHRISTENSEN, P. A. TRESCO
Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: It is widely believed that the brain tissue foreign body response (FBR) is a significant challenge for chronic single unit recording using silicon microelectrode arrays; however, few studies have directly examined this hypothesis. Previously, we found that the spatial intensity of inflammation-associated biomarkers inversely correlated with Utah Electrode Array (UEA) recording performance when one examines the FBR to the entire device in rat cortex. In this study we looked more closely at the spatial distribution of such biomarkers to the recording performance of individual microelectrodes within 4X4 UEAs that were implanted into the motor cortex of young adult male Sprague-Dawley rats (N=25). Every week after implantation, recordings were obtained from awake, unrestrained animals. At various time points and levels of functionality, rats were perfused transcardially and their brains post-fixed for 24 h in 4% paraformaldehyde. The relative intensity and spatial distribution of cell nuclei, the astrocyte cytoskeleton, neuronal nuclei, neuronal processes, macrophages, activated macrophages, blood-brain barrier leakage, myelin, and axons were evaluated using immunohistochemical methods. Initially, the signal to noise ratio (SNR) of isolated action potentials was not significantly different between the outer 12 microelectrodes and the inner 4 microelectrodes. However, by the final session, SNR decreased by 16% ($p = .13$) for the outer electrodes and 45% ($p = .02$) for the inner electrodes, resulting in significantly higher SNR for outer electrodes at the final session ($p = .04$). Quantification of immuno-labeling revealed that microelectrodes along the edge of the array had significantly lower levels of IgG and CD68 within the presumptive recording zones compared with microelectrodes located in the center of the array. Using a 3-D visualization perspective, we observed large volumes of damaged, inflamed tissue located towards the center and base of the array. Microelectrodes in these tissue areas generally performed more poorly: 12% of electrodes in damaged areas recorded at least one single unit during the device's lifetime, compared to 27% for other electrodes ($p = .0012$), and 1% of electrodes in damaged areas recorded single unit activity just before sacrifice, compared to 10% for the other electrodes ($p = .016$). These results indicate that recording performance correlates with spatial patterns of FBR biomarkers, and suggest that microvascular damage of penetrating arterioles in rat cortex plays a major role in determining the biocompatibility of high density silicon microelectrode recording arrays implanted in rat cortex.

Disclosures: **N. Nolta:** None. **M.B. Christensen:** None. **P.A. Tresco:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 445.02/LL24

Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-11-1-4120

Title: Chronic single unit recordings from UEA's implanted in aged rat cortex

Authors: ***M. B. CHRISTENSEN**, N. F. NOLTA, P. A. TRESCO
Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Implantable neural recording arrays have shaped our understanding of how the brain works, and, more recently, are being developed to treat a variety of CNS disorders. The development of long lasting clinically useful devices may be challenging in the aging population where neuroinflammatory sequela may be exacerbated in the aged brain. The goal of this study was to examine whether it was possible to obtain recordings from clinical style recording arrays implanted in the aged rat cortex over a chronic time period. We implanted 4x4 Utah Electrode Arrays (UEAs) obtained from Blackrock Microsystems (SLC, Utah) into the cortex of aged, obese male Sprague-Dawley rats (at least 70 weeks old at the time of implantation), who were aged in-house for the purposes of this study (N=8). Several died of natural causes or were removed due to the development of foot ulcers. One week after UEA implantation, and at least weekly thereafter, electrophysiological recordings were obtained from awake, unrestrained animals and analyzed offline for single unit activity. Animals were sacrificed 12 weeks after implantation for histological analysis. Robust isolated single units were obtained over the 12-week implantation period, with the maximum number of units obtained from a single animal in a single week being 21. The average number of units per animal peaked around 4 weeks (~10 units per animal) and declined thereafter until sacrifice (to a low of ~2 units per animal). As has been described with younger cohorts, some units were consistently observed week-to-week, while many units appeared variable. This study is the first of its kind to provide information regarding chronic recording function in freely moving aged obese rats. Our results indicate that is it possible to obtain robust recording performance in the aged cortex over a 3 month indwelling period in rat. A detailed histological analysis of the cohort will be reported at the meeting.

Disclosures: M.B. Christensen: None. **N.F. Nolta:** None. **P.A. Tresco:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 445.03/LL25

Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-10-C-2008

Title: One size does not fit all: Calibrating microstimulation to individual subjects using spiking network models

Authors: ***C. KERR**[1,2], J. S. CHOI[1], S. DURA-BERNAL[1], J. T. FRANCIS[1], W. W. LYTTON[1]
[1]Physiol. and Pharmacol., State Univ. of New York, Brooklyn, NY; [2]Univ. of Sydney, Sydney, Australia

Abstract: Microstimulation is an effective tool for manipulating brain activity, but its drawbacks are sobering: electrode locations are not known exactly, electrode efficacy is variable, the cells being stimulated are rarely those being recorded from, and the number of independent electrodes is much smaller than the dimensionality of the systems being stimulated. One approach is to use optimal control algorithms to design microstimulation protocols through trial and error. However, the effectively infinite space of stimulation protocols, the slowness of training optimal control models, and the limited lifespan of experimental subjects make it unlikely that such approaches could find the globally optimal solution. Computer simulations, by contrast, are omniscient and immortal; their sole limitation is their potential dissimilarity to real brains. In this pilot study, we show how a spiking network model can be used with optimal control algorithms to expedite the development of microstimulation protocols. Specifically, we tuned and validated large-scale spiking network models against data from individual rats, then used these calibrated models to design microstimulation protocols specific to each subject. Electrophysiological data were recorded from the somatosensory cortex and thalamus of five rats during both natural touch and microstimulation. The network models consisted of 24,000 spiking Izhikevich neurons with cell types and connectivities drawn from empirical data. Global model parameters (including overall balance of excitation versus inhibition, connection probability, average axon length, and relative strength of internal versus external input) were calibrated to experimental data (including firing rates, local field potential [LFP] spectra, peristimulus time histograms, stimulus fields, and inter-electrode mutual information) using a nonlinear optimization algorithm called Bayesian adaptive locally linear stochastic descent. Once tuned, the spiking network models were used with a model predictive control (MPC) algorithm to design a microstimulation protocol that minimized mismatch with an output LFP. Compared to applying MPC to a subset of simulation data comparable to that actually available from the subjects, applying it to the full set of data available in the simulation significantly reduced prediction error. Furthermore, the microstimulation protocols tuned to individual brains showed less error than protocols designed

using pooled data only. These results demonstrate the potential of using spiking network models calibrated to individual brains for the development of microstimulation protocols.

Disclosures: C. Kerr: None. **J.S. Choi:** None. **S. Dura-Bernal:** None. **J.T. Francis:** None. **W.W. Lytton:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

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Topic: D.18. Brain-Machine Interface

Support: NIH R25NS080687

Title: Tanycytic Ependymoma in a Puerto Rican 76 years old male. Ependymoma is a generally slowly growing tumor of children's and young adults originating from the wall of the ventricles or from the spinal canal, and it is composed of neoplastic ependymal cells. They correspond to a WHO Grade II tumor. Tanycytic Ependymoma is a rare variant of Ependymoma usually arising in the intra medullary spine. They have a unique morphology with a close resemblance to Schwannoma and some Astrocytoma's. We present a case of a 76 years old male with a progressive paraparesis for 8 years, due to a spinal tumor. The tumor was classified as Tanycytic Ependymoma. This is a challenging diagnosis since this lesion can be confused with other tumors. Therefore, it is important to be aware of this variant of Ependymoma and its immunohistochemistry profile. To our knowledge this is the oldest patient known to have this rare tumor. It is also the first case reported in Puerto Rico

Authors: *Y. ORTIZ[1,2], I. VEGA[3], J. L. PEREZ[4]
[1]Neuro ID, Bayamón, Puerto Rico, Puerto Rico; [2]Natural Sci. and maths, Interamerican Univ. of Puerto Rico, Bayamon, PR; [3]Biol. Dept., Univ. of Puerto Rico, Rio Piedras, PR; [4]Dept. of Pathology, Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR

Abstract: Ependymoma is a generally slowly growing tumor of children's and young adults originating from the wall of the ventricles or from the spinal canal, and it is composed of neoplastic ependymal cells. They correspond to a WHO Grade II tumor. Tanycytic Ependymoma is a rare variant of Ependymoma usually arising in the intra medullary spine. They have a unique morphology with a close resemblance to Schwannoma and some Astrocytoma's. We present a case of a 76 years old male with a progressive paraparesis for 8 years, due to a spinal tumor. The

tumor was classified as Tanycytic Ependymoma. This is a challenging diagnosis since this lesion can be confused with other tumors. Therefore, it is important to be aware of this variant of Ependymoma and its immunohistochemistry profile. To our knowledge this is the oldest patient known to have this rare tumor. It is also the first case reported in Puerto Rico.

Disclosures: **Y. Ortiz:** None. **I. Vega:** None. **J.L. Perez:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

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CWRU Start-up

PECASE

APT Center

Swiss NSF 406240_126046

Title: Antioxidant-releasing mechanically-compliant polymers to attenuate the neuroinflammatory response at the microelectrode-tissue interface

Authors: ***J. K. NGUYEN**[1,2], K. BUCHANAN[1,2], M. JORFI[3], E. FOSTER[3], C. WEDER[3], J. R. CAPADONA[1,2]
[1]Case Western Reserve Univ., Cleveland, OH; [2]APT Ctr., Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [3]Adolphe Merkle Inst., Univ. of Fribourg, Marly, Switzerland

Abstract: The decline in performance of intracortical microelectrodes can be directly linked to the foreign body response to the implanted device. A variety of factors contribute to the neuroinflammatory response to microelectrodes. Therefore, a combined approach addressing various aspects of the response is needed to improve material design and long-term performance.

Previously, our lab has reported on the efficacy of two natural antioxidants, resveratrol and curcumin, in reducing neuroinflammation at acute time points by targeting TLR4-mediated activation of NF-κB pathways. Additionally, compliant polymer implants were shown to reduce the chronic neuroinflammatory response by decreasing the mechanical mismatch between traditionally stiff microelectrode materials and neural tissue. Therefore, we hypothesize that a synergistic approach utilizing local release of antioxidants from compliant polymers implants could reduce neuroinflammation during acute and chronic neurodegenerative onsets. To test our hypothesis, *in vitro* assays were performed to test cell toxicity, drug release profiles, and antioxidant activity for various antioxidant concentrations released from compliant polymer materials. After determining optimal release, adult male rats were implanted with antioxidant releasing polymers in the cerebral cortex for acute and chronic time points of inflammation. Immunohistological expression of neurodegenerative factors, including oxidative stress, microglial/astrocytic cells and neuronal cell death, were quantified up to 500 µm away from the interface. *In vivo* experiments at two weeks post-implantation show that resveratrol-releasing polymer implants are able to improve neuronal survival (Fig 1) and reduce microglial activation. Additionally, antioxidant releasing polymers reduced reactive oxygen species and blood brain barrier permeability around the implantation site. *In vivo* experiments at later time points are currently underway. Future studies will correlate the reduction in inflammatory response to functional neuronal recordings.

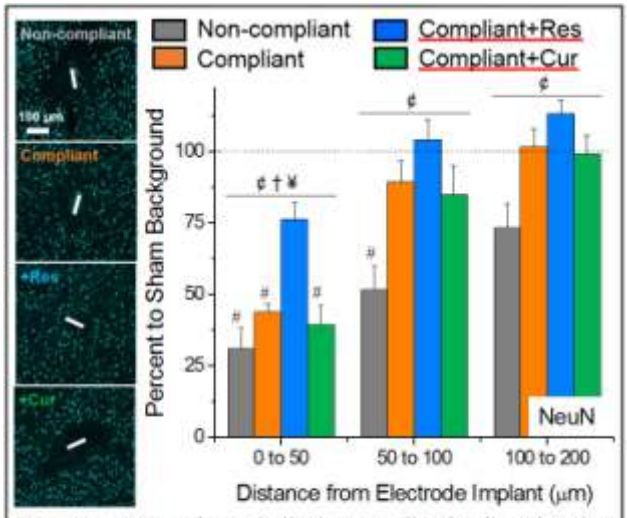

Figure 1. Neuronal populations surrounding implanted probes *in vivo* two weeks post-implantation. Polymers releasing resveratrol improved neuronal survival, compared to non-compliant controls, up to 200 µm from implant. Statistical significance # vs. Sham, ¢ Non-compliant vs. Compliant+Res, † Compliant vs. Compliant+Res, ¥ Compliant+Res vs. Compliant+Cur ($p<0.05$).

Disclosures: **J.K. Nguyen:** None. **K. Buchanan:** None. **M. Jorfi:** None. **E. Foster:** None. **C. Weder:** None. **J.R. Capadona:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

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Topic: D.18. Brain-Machine Interface

Support: University of Wisconsin Graduate School

Wisconsin Alumni Research Foundation

Title: Chronic *in vivo* imaging of sciatic nerve via a peripheral nerve window

Authors: M. HAYAT, S. BRODNICK, S. KAPUR, K. ELICEIRI, L. KRUGNER-HIGBY, S. POORE, *J. C. WILLIAMS
Biomed. Engin., Univ. of Wisconsin, MADISON, WI

Abstract: *In vivo* imaging is an important tool adopted by many scientific fields for the purpose of visualizing and documenting what is happening in a working biological system. The challenges of adopting these technologies are great, but the extensive amount of information that can be collected from minimally disturbed properly functioning systems is an invaluable asset to researchers. Currently, there have not been any studies to date that have used a chronic imaging preparation in the peripheral nervous system (PNS), that do not involve invasive opening and closing of wounds, to observe which biological processes are occurring during normal PNS function, or more importantly what occurs during pathological phenomenon. In order to move past current practices and access portions of the nervous system which are currently unavailable over a period of time longer than one day, we have developed a method that would safely and effectively raise the peripheral nerve into viewing range, and fabrication and implantation of a device which would allow for chronic imaging of the nerve in view. We have investigated the use of an implantable device with attached optically clear window for use in chronic investigations into the normal and pathological functioning of peripheral nerves. The window is a multi-component tool that is created from a metal tower, which prevents tissue growth over the window, and two bases made from polydimethylsiloxane (PDMS), and acrylonitrile butadiene styrene (ABS). The metal tower attaches to the first ABS base, which has an attached quartz glass window, creating a device to directly suture and fixate into nearby tissue of the nerve of interest. An additional PDMS device has been molded to simultaneously embed the nerve and connect to the other ABS base. Initial chronic *in vivo* studies were done to observe vascularization of the nerve post-surgery.

Disclosures: **M. Hayat:** None. **J.C. Williams:** None. **S. Brodnick:** None. **S. Kapur:** None. **K. Eliceiri:** None. **L. Krugner-Higby:** None. **S. Poore:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

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Program#/Poster#: 445.07/MM1

Topic: D.18. Brain-Machine Interface

Support: NIH NS072651

Title: Cell counts in the vicinity of implants show more neurons surrounding braided microprobes than single 50um wire-electrodes in rats' brains at 8 weeks post-implant

Authors: ***T. KIM**[1], Y. ZHONG[2], A. BRANNER[1], S. F. GISZTER[1,2]
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Sch. of Biomed. Engineering, Sci. and Hlth. Syst., Drexel Univ., Philadelphia, PA

Abstract: At SFN 2013 we showed the immune marker comparisons between implanted braided microprobes and single 50um wires as a reference electrode in rats' brains and spinal cords. In the comparison, braided microprobes showed less immunoreaction than 50um wires, and this was quantified by relative fluorescent intensity analysis with GFAP and ED1 staining. As a paired analysis, we have now performed cell counting in the vicinity of intracortically implanted electrodes with NeuN stained sections in order to observe if there is any difference in the number of surrounding neurons among the electrode designs. All tissue sections used for this analysis are some of sections made from the experiment for last year's immunohistological comparison. Therefore except analytical methods, all experiment conditions are identical with the last year's and following: 4 different chronic implants per rat (12 count 9.6um nichrome wire braids and single 50um nichrome wires in 2 different configuration, without tether and with tether anchored to the skull) and total 8 rats for 8 weeks. This design allows us to compare the tissue reaction to the different compliances of two electrode bodies and the tissue reaction to the different compliances of two tethers at the same time. For cell counting, we developed a custom Matlab GUI based code which combines automatic blob detection techniques to identify cell bodies with techniques of manually adding/removing cells by visual inspection to correct the errors caused by the automation. The vicinity of the electrode was divided into 12 annular subareas by 25um radial increments up to 300um from the contour of electrode surface. Cell density in 100x100 pixels, normalized with the cell density in the large reference area to estimate the average cell

density in each section, was compared in each subarea. Results showed that the cell density within a 100um radius from the braid surface was higher than within a 100um radius from the 50um wire surface, regardless of the tethering (i.e., anchored status to the skull). Two way ANOVA showed that the 4 different electrodes types were significant different in cell density in subareas, but were not significantly different among rats. These results strongly support our hypothesis that braided microprobes cause not only less immune responses, but also promote neurons viability and continued presence in the vicinity of implants.

Disclosures: **T. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents Pending. **Y. Zhong:** None. **A. Branner:** None. **S.F. Giszter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents pending.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 445.08/MM2

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Analysis of dopamine and serotonin under 2 min to further improve time resolution in on-line microdialysis

Authors: *M. EYSBERG, L. M. VAN HEERWAARDEN, H.-J. BROUWER, N. REINHOUD
Antec BV, Zoeterwoude, Netherlands

Abstract: Microdialysis of neurotransmitters *in vivo* has become an invaluable tool to study neurotransmission in the living brain. Extracellular fluid of the brain is sampled via a microdialysis probe and fractions are collected for further analysis. Typical flow rates in microdialysis are 1 - 2 µL/min, and decreasing the fraction size to a few microliters enables a temporal resolution of a few minutes. However, this would also require an analytical system that has the sensitivity for reliable quantification of neurotransmitters and the capability to handle samples of only a few microliters. In case of on-line analysis, the sample fractions are collected in a sample loop and analyzed immediately. For uninterrupted analysis in such a setup, the UHPLC analysis time should 'match' the time required to collect a sample. We developed a fast and reliable method for analysis of dopamine (DA) and serotonin (5-HT). Small samples of less than 2 uL were analyzed on a UHPLC system with a new electrochemical detector and a new

flow cell, the DECADE Elite and Sencell. DA and 5-HT were quantified in less than 2 min total analysis time. An increased data rate was applied to analyze the fast chromatographic peaks and an elevated column temperature was applied to further facilitate the speed of separation. In the Sencell a proprietary Adjustable Spacer Technology (AST) is applied. The principle and feasibility of this set-up is shown with the analysis of dopamine (DA) and serotonin (5-HT), which showed a detection limit of about 0.2 nmol/L (1 uL injection so about 0.2 fmol on column), with a temporal resolution of about 1 min.

Disclosures: M. Eysberg: None. **L.M. van Heerwaarden:** None. **H. Brouwer:** None. **N. Reinhoud:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 445.09/MM3

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH

Title: Cognitive dysfunction and augmented cellular autofluorescence in schizophrenia

Authors: *T. TSUJIMURA[1], A. RAMOS[1], C.-Y. LIN[1], T. SAITO[2], F. EMILIANI[1], J. GALLEGO[3], X. INDURKHYA[4], N. GAMO[1], M. KOGA[1], T. MASEDA[1], T. SEDLAK[1], Y. HORIGUCHI[5], K. TAGUCHI[5], A. MALHOTRA[6], C. KORTH[4], K. ISHIZUKA[1], A. SAWA[1]
[1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Aomori Univ., Aomori, Japan; [3]Hostra North Shore LIJ Sch. of Med., Hempstead, NY; [4]Heinrich Heine Univ., Düsseldorf, Germany; [5]Showa Pharmaceut. Univ., Tokyo, Japan; [6]The Zucker Hillside Hosp., Glen Oaks, NY

Abstract: We discovered that the levels of cellular autofluorescence (AF) are significantly elevated in lymphoblasts from 46 schizophrenia patients (SZ) compared with those from 38 matched controls. Sub-chronic treatment of the lymphoblasts with antipsychotics did not alter the levels of AF. AF in lymphoblasts obtained from drug naïve patients and the same patients after one-year of medication were unchanged, indicating that increased AF is not likely to be a consequence of medication. Several groups, including ours, have reported increased oxidative stress associated with SZ. Interestingly, the aberrantly elevated AF was found to be correlated with cellular levels of reactive oxygen species. Furthermore we found a specific correlation between the levels of AF and cognitive impairment, but not with positive or negative symptoms

in SZ. Given that cellular AF is a promising marker for oxidative stress and cognitive changes in SZ, we further investigated these cellular and behavior phenotypes in animal models. In two independent animal models, Eaac1 knockout mice and dominant-negative DISC1 transgenic mice, that display augmented oxidative stress and aberrant behaviors relevant to SZ, we observed increased AF levels in brain tissues from these mice, which are consistent with the observations in SZ lymphoblasts. We also obtained preliminary but promising results that a specific inhibitor for the GAPDH stress signaling pathway could improve abnormal behavior in these mice. Molecular mechanisms underlying the elevated AF in SZ is now being investigated through the GAPDH stress signaling pathway (see the abstract by Adriana Ramos et al, SFN 2014).

Disclosures: **T. Tsujimura:** A. Employment/Salary (full or part-time):; Dainippon Sumitomo Pharma Co., Ltd.Dai. **A. Ramos:** None. **C. Lin:** None. **T. Sedlak:** None. **K. Ishizuka:** None. **F. Emiliani:** None. **N. Gamo:** None. **T. Maseda:** None. **M. Koga:** None. **A. Sawa:** None. **J. Gallego:** None. **T. Saito:** None. **X. Indurkhya:** None. **Y. Horiguchi:** None. **K. Taguchi:** None. **A. Malhotra:** None. **C. Korth:** None.

Poster

445. Brain–Machine Interface: Analytical Methods for Monitoring Tissue Responses

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 445.10/MM4

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: SBT Award W32TAG

Title: Breath analysis for biomarkers of toxicant exposure

Authors: *A. B. MANNING-BOG[1], R. D. LEIB[3,2], J. D. WHITE[2]
[1]Ctr. for Hlth. Sci., [2]Sensor Systems Lab., SRI Intl., Menlo Park, CA; [3]Vincent Coates Fndn. Mass Spectrometry Lab., Stanford Univ., Stanford, CA

Abstract: Environmental toxicant exposure is a significant risk factor for the development of disease, including neurodegenerative disorders such as Parkinson's disease (PD). Recent epidemiological studies in twin pairs have revealed a 6-fold increase in the incidence of PD in individuals exposed to trichloroethylene (TCE). Motor symptoms are most commonly used for diagnosis of PD and manifest after significant degeneration of the nigrostriatal pathway.

However, the pathophysiological mechanisms that underlie the disorder are known to initiate much earlier. Assessment of biomarkers following toxicant exposure has the potential to not only measure exposure and identify at-risk populations, but also possibly detect the disease in its earliest stages. We are developing a non-invasive breath test to measure volatile organic compounds (VOCs) for monitoring of environmental exposures and evaluation of VOCs potentially indicative of nigrostriatal degeneration. Our preliminary studies utilize exhaled samples from a pre-clinical mouse model (C57BL/6 males) exposed systemically to TCE. After i.p. administration of TCE or saline, breath environment samples were collected from each mouse over time and analyzed using laser ionization time-of-flight mass spectrometry. This approach provides high sensitivity with a very short analysis time and can identify unknown VOC formulae in the absence of external standards. Our preliminary results indicate that we can detect both TCE and other VOCs that may be indicative of neurodegeneration in the breath after systemic exposure to the toxin. On-going studies are evaluating the potential relationship between breath VOCs and known neurochemical and biochemical markers of nigrostriatal damage in animal models, and (critical to patients) correlating altered VOCs from the pre-clinical models with human PD breath samples.

Disclosures: A.B. Manning-Bog: None. **R.D. Leib:** None. **J.D. White:** None.

Poster

446. Comparative Anatomy and Evolution I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 446.01/MM5

Topic: D.19. Comparative Anatomy and Evolution

Support: FAPESP 2012/08833-9

FAPESP 2012/03067-6

Title: Distribution of teneurin-4 immunoreactivity in the central nervous system of non-human primates (Sapajus spp)

Authors: *K. R. TORRES DA SILVA[1,2], A. V. DA SILVA[2,3], J. A. DE OLIVEIRA[1], E. ERVOLINO[1], A. GONÇALVES[1], J. C. BITTENCOURT[4], C. A. CASATTI[1,2]
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Sul/ UFMS, Tres Lagoas, Brazil; [4]Biomed. Sci. Inst. of Sao Paulo University/ USP, Sao Paulo, Brazil

Abstract: Teneurins are a family of four transmembrane proteins (Ten-1 to -4) highly conserved among D. melanogaster, *C. elegans* and vertebrates. The main sites of expression are in the neuronal tissue, exerting an important role during neurogenesis and transcriptional regulation. Neuroanatomical studies analyzing teneurins have shown that their expressions are quite similar in some regions of zebrafish, chicken and mice, mainly during neuronal development of the central nervous system (CNS). However, the neuroanatomic distribution of teneurins in the adult primate brain has not been revealed yet. The present study analyzed Ten-4 immunoreactivity distribution in the CNS of three adult Cebus monkey (Sapajus spp) using immunoperoxidase method developed with diaminobenzidine-nickel. All procedures are according with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and approved by the Institutional Committee on Animal Research and Ethics. Ten-4 immunoreactive perikarya were noted in the hippocampus (mainly CA2), amygdalohippocampal area, putamen, subthalamic nucleus, ventral part of thalamus, dorsal lateral geniculate nucleus, oculomotor nucleus, Edinger-Westphal nucleus, purkinje cells and deep nuclei of cerebellum, olivar inferior complex, external cuneate nucleus, cochlear nuclei and scattered cells in the lateral hypothalamic area. Immunoreactive nerve fibers were present in the amygdalopiriform transition area, basal nucleus (Meynert), accumbens nucleus, substantia innominata, island of Calleja, ventral pallidum, ventral and dorsal part of lateral septal nucleus, external globus pallidus, anteromedial thalamus, spinal trigeminal tract, dorsal reticular nucleus and solitary tract. The data showed that Ten-4 immunoreactivity is preserved in the primate brain and based on its distribution it can be involved in different brain functions, such as motor, sensory and autonomic control as well as in emotion and memory modulation.

Disclosures: K.R. Torres Da Silva: None. **A.V. da Silva:** None. **J.A. de Oliveira:** None. **E. Ervolino:** None. **A. Gonçalves:** None. **J.C. Bittencourt:** None. **C.A. Casatti:** None.

Poster

446. Comparative Anatomy and Evolution I

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Program#/Poster#: 446.02/MM6

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH grant R01NS035103

Title: Revealing functional organization of frontoparietal networks in tree shrews (Tupaia belangeri) using reversible inactivation

Authors: *M. K. BALDWIN[1], D. F. COOKE[1], A. GORDON[1], L. A. KRUBITZER[2]
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Abstract: Tree shrews are the closest living relative to primates, yet only a few studies have examined the organization of motor and parietal cortex in this species. Such studies have revealed that tree shrews share many similar connectional patterns between frontal and parietal cortex with primates; however, an understanding of the functional organization of this frontoparietal network is lacking. Previously, it has been shown that that simple movements can be elicited with short train intracortical microstimulation (ICMS) of primary (M1), and secondary (M2) motor cortex, as well as primary somatosensory (S1), secondary somatosensory (S2), parietal ventral (PV), 3a, and caudal somatosensory (Sc) areas in tree shrews, all of which project directly to the spinal cord (Remple et al., 2006). Additionally, M1 and M2 share connections with Sc and other posterior parietal (PPC) domains, but do not share direct connections with primary somatosensory cortex. In the present study, we investigated the functional organization of motor and parietal cortex using long train (LT) ICMS coupled with reversible inactivation via cooling of motor cortical areas in an effort to appreciate how the simple frontoparietal network in tree shrews may have given rise to the more complex networks found in primates. We hypothesized that cortical areas that are directly connected would influence the neuronal properties of each other to a greater extent than areas that were not a part of these frontoparietal networks. To test this idea, we systematically reversibly inactivated M1 and M2 using a microfluidic cooling device, and determined if LT-ICMS-evoked movements were altered in 3a, S1, S2/PV, and Sc/PPC. The most common results of cooling M1 and M2 included higher current thresholds for stimulation-evoked movements, along with movement trajectories that were truncated. Specifically, inactivation of M1 greatly reduced or abolished movements evoked from Sc/PPC, and 3a, but had minimal effects on movements evoked from S1. Thus, movements in Sc/PPC, and 3a are greatly influenced by M1/M2, whereas movements evoked from S1 are independent, or minimally influenced by M1/M2. Our findings in the tree shrew are similar to those observed in New World and prosimian primates using muscimol inactivation techniques (Stepniewska et al., 2014) and suggest that there are organizational and functional characteristics of the frontoparietal motor network that are conserved across these species, and were likely present in their common ancestor.

Disclosures: M.K. Baldwin: None. **A. Gordon:** None. **L.A. Krubitzer:** None. **D.F. Cooke:** None.

Poster

446. Comparative Anatomy and Evolution I

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Topic: D.19. Comparative Anatomy and Evolution

Support: NSF grant SMA 1041755 to GWC

Center for Academic Research and Training in Anthropogeny (CARTA) Fellowship to BC

Title: Interhemispheric functional connectivity is not selectively reduced in larger-brained species

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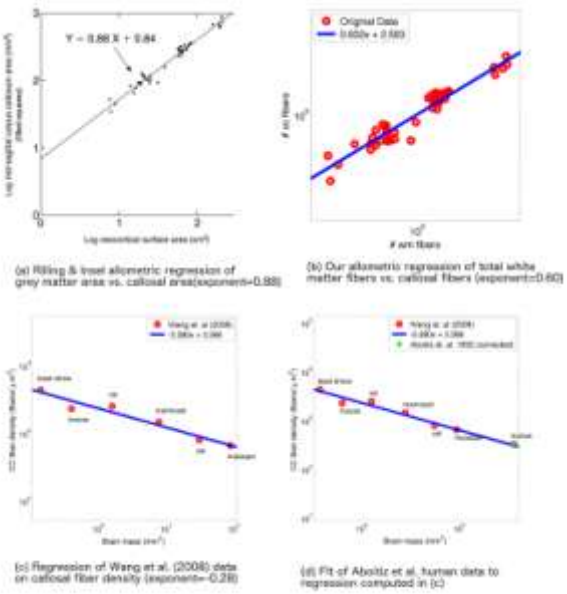
Abstract: Rilling & Insel (1999) argued that in primates, interhemispheric connectivity is selectively reduced as a function of brain size, leading to reduced functional connectivity in larger brains. They compared callosal mid-sagittal area and grey matter surface area to estimate connectivity, which ignores cross-species scaling effects for callosal fiber density and grey matter neuron density. We mined data from the literature to estimate inter- and intrahemispheric connectivity directly. We find that (1) the proportion of total connectivity that is interhemispheric is much more drastically reduced as a function of brain size than previously reported (Fig.1a and 1b), but that (2) this reduction is not selective for interhemispheric connections: axon counts per fiber tract interconnecting two cortical areas scale similarly for inter- and intrahemispheric connections. This is due to the increasing number of area-area intrahemispheric fiber tracts as brain size increases (Changizi & Shimojo, 2005) compared to the largely homotopic interhemispheric connections. Thus, any claim of a reduced role for interhemispheric connections would also have to claim that, e.g., V1=>V2 is less functionally relevant. On the contrary, we estimate the average interhemispheric area-area connection contains 3x-8x more fibers than the average intrahemispheric one, despite on average being longer--suggesting a special role for interhemispheric connections that persists over all brain sizes. To obtain these results, we computed an allometric regression of callosal fiber density using data from Wang et al. (2008; Fig 1c). We also computed the human callosal fiber density using the best available data (Aboitiz et al., 1992), correcting for several confounds (including a novel correction for lifespan differences between the human and non-human samples), and found it to be consistent with the Wang data (Fig. 1d). Our methods yield an estimate of ~220-240 million fibers in the human corpus callosum, 20% more than previously reported (Aboitiz et al., 1992).



Disclosures: **B. Cippolini:** None. **G.W. Cottrell:** None.

Poster

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Program#/Poster#: 446.04/MM8

Topic: D.19. Comparative Anatomy and Evolution

Title: All cortices fold the same: Gyrification as a universal function of cortical surface area, not number of neurons

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Abstract: Gyrification, the formation of sulci and gyri in the cortical surface, is a prominent feature that accompanied cortical expansion in evolution. Although a basic property of the cerebral cortex, gyrification remains ill understood, despite a number of proposals relating it to brain volume, cortical thickness, cortical connectivity through the white matter and to the number of cortical neurons. If the degree of cortical folding results from a single developmental mechanism, a systematic analysis of its variation across a large sample of mammalian species

should be able to retrieve at least one universal relationship valid across species and clades. Here we examine 2 datasets in search for such a unifying scaling of cortical folding. One is our own, consisting of 25 species spread over 6 clades. The second dataset consists of data published by other groups on cortical surface area, thickness, brain volume and folding index for 43 mammalian species, yielding a total sample of 64 species. We find that cortical folding can not be predicted from either brain mass or cortical neuron number. In contrast, it is well described by a power law function of the total surface area of the cerebral cortex, that holds for three orders of magnitude. This strongly suggests the existence of a universal scale-invariant mechanism for cortical folding We propose here a universal model based on the observed orthogonal crossing structure of axonal fiber bundles in white matter and the measured plastic response of axons to external forces. The later allows us to define an effective WM 'energy', the minimization of which naturally segregates axons into shallow and deep fibers, leading to the formation of sulci in a self-similar way. Using a statistical physics analysis that includes the effects of cortical surface self-avoidance and other surface terms, we correctly compute the exponent relating folding and cortical area, as well as the transition between gyrified and lyssencephalic cortices. The finding that the degree of gyrification of the cerebral cortex across diverse mammalian species can be explained simply through the self-avoiding folding of the cortical surface due to a anysotropic distribution of connectivity through white matter has important implications for the developmental origin of cortical folding in evolution: it implies that folding forms according to the same physical, tension-based mechanism regardless of the cellular mechanisms that cause the cortical surface to expand in each species, and therefore regardless of the numbers of neurons that form the cortex. Thus, our findings imply that there is probably not a genetic mechanism that controls cortical folding per se.

Disclosures: **B.C. Mota:** None. **S. Herculano-Houzerl:** None. **J. Gomes:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: CNPq

FAPERJ

James S. McDonnell Foundation

Title: Human and non-human primates have similar distributions of neurons along the cerebral cortex, including prefrontal cortex

Authors: ***M. GABI**[1], K. NEVES[1], C. MASSERON[1], P. RIBEIRO[1], L. VENTURA-ANTUNES[1], J. H. KAAS[2], S. HERCULANO-HOUZEL[1]
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Abstract: Brain evolution is often considered synonymous with expansion of the cerebral cortex, the structure to which our remarkable cognitive skills are often attributed. Human evolution in particular is thought to have involved a selective enlargement of the volume of the prefrontal cortex, although this popular notion has recently been challenged (Barton and Venditti, 2013). Cortical volume, however, is not necessarily a good proxy for numbers of neurons, which must be compared directly across species and cortical areas (Herculano-Houzel, 2011). Here we determine if the expansion of numbers of cortical neurons in primate evolution, and human evolution in particular, occurred in a homogenous manner across the cortex or was, for instance, accompanied by an increase in the relative number of neurons in the prefrontal cortex. We analyzed the distribution of neurons along the anteroposterior axis of the cerebral cortex of seven non-human primate species (Saguinus midas, Otolemur garnetti, Macaca fascicularis, Aotus trivigatus, Papio papio, Cebus apella, Macaca nemestrina) and one human cortex (Ribeiro et al., 2013). Each brain had one cortical hemisphere cut completely into a series of 2 mm coronal sections, each of which had its number of neurons determined using the isotropic fractionator (Herculano-Houzel and Lent, 2005). We find the distribution of neurons along the A-P axis of the cerebral cortex to be skewed towards occipital areas in all 8 species, departing from the distribution of gray matter volumes. This is due to up to 5-fold larger neuronal densities in occipital areas than in frontal areas, matching findings that average neuronal size is largest in the primate prefrontal cortex (Elston et al., 2001). Most importantly, the prefrontal cortex, defined as all cortex anterior to the corpus callosum, holds approximately 8% of all cerebral cortical neurons in all 8 species, despite a variance in cortical volumes of 40-fold across species. The relationship between gray matter volume and number of neurons across species differs for prefrontal, occipital and intermediate regions of the cortex, but the scaling exponents are similar across the three regions, without any preferential expansion of prefrontal areas. These findings indicate that human evolution occurred with no particular increase in the relative distribution of cortical neurons towards prefrontal areas and thus no selective expansion of the number of neurons in prefrontal cortex. Still, the same 8% of all cerebral cortical neurons correspond to a much larger absolute number of neurons in human than in other primate brains, which could explain our superior cognitive abilities compared to other primates.

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Poster

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Support: CNPq Postdoc Fellowship

Title: Cellular scaling rules for marsupial brains

Authors: *S. E. DOS SANTOS[1], J. PORFIRIO[1], F. BARROS DA CUNHA[1], M. A. RAGHANTI[2], C. C. SHERWOOD[3], S. HERCULANO-HOUZEL[1]
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Abstract: Brain size varies over a hundred thousand times in mammalian evolution, with cortices ranging from small and lyssencephalic to very large and gyrified, to which complex cognitive abilities are attributed. Understanding the rules that govern the relationship between the mass of different brain structures and the numbers and proportions of the different cell types that compose them is essential to understand how brains are made and how they evolved. While common scaling rules apply to the non-neuronal composition of the brain of all eutherian mammals analyzed so far, neuronal scaling rules differ in some clades. The same neuronal scaling rule is shared by the cerebral cortex of afrotherians, artiodactyls, rodents and eulipotyphlans, but not by primates, whereas neuronal scaling rules for the cerebellum are shared by the 3 first clades, but not by eulipotyphlans and primates. These findings suggest that the ancestral scaling rules for mammalian brains are those that are shared by extant afrotherians, artiodactyls, eulipotyphlans and rodents. Marsupials, a clade that emerged early, prior to modern eutherians, are a key group to test the putative ancestrality and universality of the non-neuronal composition of the mammalian brain, and also the presumed ancestral neuronal scaling rules inferred from extant eutherians. If marsupials are found to conform to the non-neuronal and neuronal scaling rules that apply to most extant mammalian species, it will be possible to infer the brain celular composition of early ancestral mammals. Here we aim to quantify the total number of neuronal and non-neuronal brain cells in marsupials. We used the isotropic fractionator to estimate the number of cells in several structures (including the cerebral cortex, cerebellum, and hippocampus, among others) and to determine how structure mass varies with number of cells. We examined the brains of 5 species (2 kangaroos and 3 wallabies), ranging in mass from 16.4g to 62.7g. This range overlaps with the distribution of brain mass in rodents,

artiodactyls and primates. Our preliminary results show that the distribution of structure mass as a function of total numbers of cells in marsupials overlaps with the distribution found previously in eutherians. The analysis of numbers of neurons specifically will show whether the marsupial brain conforms to the neuronal scaling rules shared by most eutherians, in which case those rules can be considered to extend to ancestral therians, or whether modern marsupials have also diverged from the rules that apply to modern eutherians.

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Poster

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CNPq

FAPERJ-Cientista Do Nosso Estado

Title: More neurons allow for less sleep which allows for bigger brains: A novel mechanism for the regulation of daily sleep need accounts for the joint evolution of increased brain size and decreased sleep requirement

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Abstract: What determines the number of hours of daily sleep that an animal needs, and how does that relate to brain size? Across mammalian species, there is a rough negative correlation between brain mass and daily hours of sleep: on one end of the spectrum are bats and eulipotyphlans, which sleep 15-19 h/day, and on the other, giraffes and elephants, which sleep 3-4 h/day. Primates, however, are an exception, with a relatively steady sleep requirement around 8-9 h/day despite widely varying brain masses, often larger than in short-sleeping artiodactyls. Although the rough correlation between daily sleep hours and brain mass has been noticed before, there has been so far no model or mechanism to account for the relationship. Here I test the hypothesis that sleep is triggered by the accumulation of metabolites during waking, and

sleep duration is proportional to the concentration of metabolites. If the accumulation of metabolites is proportional to the density of neurons (DN) underneath the cortical surface, and only the surface is subject to metabolite clearance during waking (Xie et al., 2013), then sleep should be triggered sooner, and last longer, in animals with the highest ratio between neuronal density and cortical surface area, DN/A, and daily sleep need should correlate positively with DN/A across species. I show that, across a set of over 20 species, DN/A is indeed the parameter that best correlates with daily sleep need, compared to several other parameters such as cortical thickness and brain mass. Remarkably, this correlation applies to primates and non-primates alike. The ratio DN/A, in turn, decreases across species in tight correlation with increasing number of neurons in the cerebral cortex, although differently across primates and non-primates. I propose that, in the evolution of non-primate mammals, the addition of neurons to the cerebral cortex led to a decrease in the DN/A ratio that allowed a slower accumulation of sleep-inducing metabolites and thus led to decreased daily sleep need. This, in turn, favored the evolution of further increases in numbers of cortical neurons by granting animals with an increased window of waking time in which to forage and obtain energy to afford more neurons (and, incidentally, a larger body as well). In primates, the addition of neurons to the cortex led to a smaller decrease in DN/A and thus increased numbers of neurons in primates were not correlated with significantly decreased sleep time. Such a link between increased numbers of neurons and decreased sleep times might explain both the trend towards increased body and brain mass in mammalian evolution and the trend towards decreased sleep times in larger-brained, non-primate mammals.

Disclosures: S. Herculano-Houzel: None.

Poster

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Title: Complex brains for complex cognition - neuronal scaling rules for bird brains

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Abstract: Many birds show remarkable cognitive abilities rivaling those observed in apes, the closest human relatives. Because absolute size of avian brains is rather small, it remains unclear how birds can accomplish this level of behavioral sophistication. Using the isotropic fractionator we determine directly the numbers of neuronal and nonneuronal cells in a total of 74 brains of adult specimens of 11 species of parrots, 14 species of songbirds and 4 selected model species representing other bird groups. We show that in parrots and songbirds the total brain mass as well as telencephalic mass scales approximately linearly with the total number of neurons, i.e. neuronal density does not change significantly as brains get larger. The neuronal densities in the telencephalon exceed those observed in the cerebral cortex of primates by a factor of 2-8. As a result, the numbers of telencephalic neurons in the brains of the largest birds examined (raven, kea and macaw) equal or exceed those observed in the cerebral cortex of many species of monkeys. The avian cerebellum features neuronal densities similar or higher than those found in primates. In contrast to primates, however, both the relative size of the cerebellum and the percentage of total brain neurons found in the cerebellum decrease in birds with increasing brain mass, from 12% to 7% and from around 50% to 20%, respectively. In the macaw brain, for instance, almost 80% of all brain neurons are contained in the telencephalon, while only 20% reside in the cerebellum, a condition reversed to what is found in mammals. By contrast, the densities of nonneuronal cells remain fairly constant regardless of brain size and brain region. These findings are congruent with data from all mammals analyzed so far, and indicate that while neuronal scaling rules for the avian brain differ from those that apply to mammalian brains, nonneuronal scaling rules are shared between the two animal classes. Finally, our findings of comparable numbers of neurons in the cerebral cortex of medium-sized primates and in the telencephalon of large parrots and songbirds (particularly corvids) strongly suggest that large numbers of forebrain neurons, and hence a large computational capacity, underpin the behavioral and cognitive complexity reported for parrots and songbirds, despite their small brain size.

Disclosures: **S. Olkowicz:** None. **M. Kocourek:** None. **R. Lucan:** None. **M. Portes:** None. **P. Nemec:** None. **S. Herculano-Houzel:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: CAPES

CNPq

Faperj

James S. McDonnel Foundation

Title: Concerted and mosaic scaling of neuronal numbers and cell size in mammalian brain evolution

Authors: *K. NEVES[1], S. HERCULANO-HOUZEL[2], P. MANGER[3], J. H. KAAS[4]
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Abstract: Enough species have now been subject to systematic quantitative analysis of the relationship between the morphology and cellular composition of their brain that patterns begin to emerge and shed light on the evolutionary path that led to mammalian brain diversity. Based on an analysis of the shared and clade-specific characteristics of 41 modern species in 6 clades of placental mammals and in light of the phylogenetic relationships among them, here we propose that ancestral eutherian brains were composed and scaled in their cellular composition like modern afrotherian and glire brains: with an addition of neurons that is accompanied by a decrease in neuronal density and very little modification in glial cell density, implying a significant increase in average neuronal cell size in larger brains, and the allocation of approximately 2 neurons in the cerebral cortex and 8 neurons in the cerebellum for every neuron allocated to the rest of brain. We also propose that in some clades the scaling of different brain structures has diverged away from the common ancestral layout through clade-specific (or clade-defining) changes in how average neuronal cell mass relates to numbers of neurons in each structure, and how numbers of neurons are differentially allocated to each structure relative to the number of neurons in the rest of brain. Thus, the evolutionary expansion of mammalian brains has involved both concerted and mosaic patterns of scaling across structures. This is, to our knowledge, the first mechanistic model that explains the generation of brains large and small in mammalian evolution.

Disclosures: **K. Neves:** None. **S. Herculano-Houzel:** None. **P. Manger:** None. **J.H. Kaas:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: CAPES

FAPERJ

James Mcdonnell Foundation

Title: Quantitative analysis of the spatial distribution of neurons, glial cells and vasculature in the mouse brain

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Abstract: Mammalian brains vary over 100,000 in volume, with neuronal densities that are highly variable across brain structures and also across species. In contrast, non-neuronal cell densities are much less variable across brain structures and species. This difference in variability is illustrated by the finding that, within our sample of 207 brain structures quantified in 40 species distributed across 6 clades, 95% of the structures have neuronal densities ranging between 95,441 and 151,527 neurons/mg, while non-neuronal densities range only between 76,073 and 89,022 non-neuronal cells/mg. Together with the finding that the relationship between brain structure mass and number of non-neuronal cells is shared across all brain structures and mammalian species examined so far, the small variation in non-neuronal cell densities points to a universal set of rules determining how non-neuronal cells are added to brain tissue and distributed within it. Non-neuronal cells however comprise two morphologically and functionally distinct populations: glial cells and endothelial cells. It is possible, therefore, that the shared scaling rules found for non-neuronal cells as a whole actually apply only to one of the two populations - or even to a particular cell type. For example, if endothelial cells were the most numerous non-neuronal cells, then the shared scaling rules might only apply to them, and not to glial cells, with wide implications for the evolutionary importance of the conserved scaling rules. While it has been estimated that the vasculature occupies no more than 4% of the cortical volume (Schuez and Palm, 1989), it remains unknown what proportion of brain cells are endothelial cells - and, therefore, what proportion of non-neuronal cells are actually glial. Here we sought to quantify these proportions in different structures of the mouse brain by using systematic random

sampling of 3D stacks of microscopic images of tissue that had blood vessels stained by a previous injection of FITC-dextran. Neurons were identified by immunocytochemistry to NeuN, and all cell nuclei. were visualized by staining with DAPI. Our results show that in the mouse cerebral cortex, 13.4% of the cells are endothelial cells, 31.3% are glial cells and 55.3%are neurons. Endothelial cells are thus more common than expected from the volume they occupy, which indicates that they are small cells compared to other cell types in the brain. Still, endothelial cells are uncommon enough that 70% of all non-neuronal cells are found to be glial cells. We can thus safely conclude that the universal scaling rules that we have found to apply to non-neuronal cells also apply to glial cells as a whole.

Disclosures: L. Ventura Antunes: None. **J. Maldonado:** None. **S. Herculano-Houzel:** None.

Poster

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Center for Intergrative Neuroscience

Title: Anterograde and retrograde examination of prefronto-insular connections in the macaque monkey

Authors: *H. C. EVRARD[1,2], J. L. PRICE[3], N. K. LOGOTHETIS[4]
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Abstract: A recent cyto- and myelo-architectonic analysis demonstrated that the classical agranular, dysgranular and granular 'sectors' of the insular cortex in the macaque monkey are divided into consistent and sharply demarcated areas, most of which contain smaller subdivisions or modules (Evrard et al., J Comp Neurol, 2014, 522:64-97). This refined architectonic map readily suggests the existence of a matching modular organization of the connections and functions of the insula. Prior injections of anterograde or retrograde tracers in distinct areas of the orbital and medial 'networks' of the prefrontal cortex (OPFC and MPFC) in the macaque

monkey labeled small discontinuous 'patches' of neurons (Saleem et al., J Comp Neurol, 2008, 506:659-93). In light of the new architectonic map, each of these patches could correspond to a distinct architectonic module. Here, we examined the modular distribution of neurons and axon terminals labeled in the insula with injections of anterograde and retrograde tracers in the distinct OPFC and MPFC network areas. Injections in OPFC areas labeled conspicuous patches of neurons or terminals in both agranular and dysgranular areas. The exact spatial location of each patch adequately matched the location of a distinct architectonic module and varied with the location of the injection site in OPFC. For example, injections in area 13m reproducibly labeled the modules '3' and '5' of the dorsal dysgranular area (Idd3 and Idd5) and the module '3' of the mound dysgranular area (Idm3), whereas injections in area 11l labeled Idm2 and Idm3 as well as Idd4 but not Idd3 and Idd5. Similarly, injections in MPFC areas produced sparser labeling that variably involved Idm2, both modules of the ventral dysgranular area (Idv1 and Idv2), the dorsal and ventral posterior agranular areas (Iapd and Iapv), and the fundal agranular area (Ivfa). The present data demonstrate that each architectonic module recently identified in the macaque insula has distinct connections with distinct OPFC and MPFC areas. This supports the view that the fine architecture of the insula provides the basis for a modular integration of interoceptive and prefrontal activities. Together with the insulo-prefrontal connections, the examination of the connections of each insular module with the rest of the cerebral cortex and with subcortical nuclei will provide a significant insight in the functional organization of the primate insular cortex.

Disclosures: H.C. Evrard: None. **J.L. Price:** None. **N.K. Logothetis:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: Max Planck Society

Title: Projections of the orbital and medial prefrontal cortex to the ventral tegmental area in the macaque monkey

Authors: *D. H. MOMBIELA[1,2], M. UBERO[2,3], J. L. PRICE[4], R. INSAUSTI[3], N. K. LOGOTHETIS[2], H. C. EVRARD[2,5]
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Tuebingen, Germany; [3]Univ. of Castilla-La Mancha, Albacete, Spain; [4]Washington Univ., Saint-Louis, MO; [5]Ctr. for Integrative Neurosci., Tuebingen, Germany

Abstract: The orbital and medial prefrontal cortex (OMPFC) in macaque monkeys sends discreet glutamatergic projections to the ventral tegmental area (VTA) (Ongür et al., J Comp Neurol, 1998, 401:480-505; Frankle et al., Neuropsychopharmacol, 2006, 31:1627-36). These projections likely provide a mild direct influence on VTA activity, in addition to a stronger indirect influence involving intermediary glutamatergic diencephalic nuclei. On the basis of its connectivity, OMPFC has been divided into orbital 'sensory' (OPFC) and medial 'visceromotor' (MPFC) networks (Price, ANYAS, 2007, 1121:54-71). Projections to VTA originate from both networks but whether their density varies across areas within a single network and whether they are topographically organized within VTA remain unknown. Here, we examined (1) the distribution of anterograde labeling produced in VTA with injections of biotin dextran amine or fluororuby in distinct architectonic areas in OPFC and MPFC, and (2) the distribution of retrograde labeling produced in PFC with injections of cholera toxin b or fluorescent dextran in VTA. The analysis of the anterograde labeling confirmed prior evidence that PFC contributes only moderate projections to VTA, in contrast with their projections to other targets (e.g. striatum). The density of anterogradely labeled fibers with varicosities in VTA varied with the location of the injection site, so that each network had areas contributing more projections than others. Injections in the medial network produced overall more labeling than injection in the orbital network. Injections in areas 25, 24b, 32, and the intermediate agranular insula (Iai) produced relatively dense labeling. In contrast, injections in areas 10o, 11m and 14c produced sparse or no labeling. In the orbital network, only injections in area 13b and in the posterior median agranular insula (Iapm) produced relatively dense labeling with no major difference between areas. Injections in all the other areas including areas 13l, 11l, 12m, 12r and 12l produced sparse or no labeling. A comparison of the spatial distribution of the labeled fibers in VTA revealed a considerable overlap of the projections from the different areas with only a subtle trend for medial projections to terminate more lateral and rostral than orbital projections. Retrograde tracers injections in VTA supported the heterogeneity of the areal distribution of the cells of origin of PFC projections to VTA. Large injections preferentially labeled areas from which dense labeling was obtained in VTA. Smaller injections tended to label only a subset of these areas supporting the existence of a discreet internal topography within VTA.

Disclosures: D.H. Mombiela: None. **M. Ubero:** None. **J.L. Price:** None. **R. Insausti:** None. **N.K. Logothetis:** None. **H.C. Evrard:** None.

Poster

446. Comparative Anatomy and Evolution I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 446.13/MM17

Topic: D.19. Comparative Anatomy and Evolution

Support: Max Planck Society

Center for Intergrative Neuroscience

Title: Insular projections to the midbrain periaqueductal gray in the macaque monkey

Authors: *T. O. SALEH[1], J. L. PRICE[2], N. K. LOGOTHETIS[3], H. C. EVRARD[1,3]
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Abstract: We recently demonstrated the presence of the large spindle-shaped von Economo neuron (VEN) in a specific architectonic area ('VEN-area') in the anterior insula in the macaque monkey (Evrard et al., Neuron, 2012, 74:482-9). Given its relatively large size and localization in layer 5a, the VEN likely projects to distant brain regions including the midbrain periaqueductal gray (PAG). A prior tracing study demonstrated that distinct areas in the macaque anterior insula project densely to PAG (An et al., J Comp Neurol, 1998, 401:455-79). Here, using previously published (An et al., 1998) and new material, we examined (1) the distribution of neurons retrogradely labeled in the insula with injections of cholera toxin b, fast blue or fluorescent dextran in different columns of PAG, (2) whether any of the architectonic areas projecting to PAG corresponds to the VEN-area, and (3) whether the VEN and its co-mingled companion 'fork' cell (FC) project to PAG. Injections in PAG invariably labeled small, discontinuous patches of neurons in the ventral portion of the insula both posterior and anterior to the limen insula. Using a recently refined architectonic map of the macaque insula (Evrard et al., J Comp Neurol, 2014, 522:64-97), we observed that the areal affiliation of these patches consistently varied with the location of the injection site. Injections in the dorsal lateral column of PAG (dlPAG) sparsely labeled the fundal agranular area (Ivfa), and the dorsal and ventral posterior agranular areas (Iapd and Iapv), posterior to the limen, and densely labeled the intermediate agranular area (Iai), anterior to the limen, as reported before by An et al. (1998). Injections in the lateral column of PAG (lPAG) labeled the 'mound' dysgranular area (Idm) and the dorsal posterior agranular area (Iapd), posterior to the limen, and the lateral agranular insula (Ial), anterior to the limen. Injections in the ventrolateral column of PAG (vlPAG) produced an intermediate labeling including both Iai and Ial. VENs and fork cells were located preferentially, if not exclusively, in Ial. An analysis of the morphology of the neurons retrogradely labeled in Ial revealed a small subset of VENs and fork cells. The projection of directly adjacent insular areas to different columns of PAG may provide a unique insight in the efferent cortical control of the autonomous system. In this context, the VEN and their companion FC could have a direct and

rapid influence on the sympathetic and parasympathetic substrate of emotional behavior and feelings.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: Max Planck Society

Title: Anterograde and retrograde analysis of the connections between the orbital and medial prefrontal cortex and the locus coeruleus in the macaque monkey

Authors: *M. UBERO MARTINEZ[1,2], D. HERNANDEZ[1,2], J. L. PRICE[3], R. INSAUSTI[2], N. K. LOGOTHETIS[1], H. C. EVRARD[1,4]
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Abstract: Prior dopamine-beta-hydroxylase immunohistochemistry suggested that the projections from the locus coeruleus (LC) to the orbital and medial prefrontal cortex (PFC) are heterogeneous (Lewis & Morrison, J Comp Neurol, 1989, 282:317-30). A tract-tracing corroboration of this heterogeneity is still lacking. In addition, whether areas of PFC that receive direct projections from LC are the same that provide modulatory feedback to LC remains unclear. Here, we examined the distribution of retrograde and anterograde labeling in LC with injections of multiple, differently-colored neuronal tracers in distinct architectonic areas in orbital and medial PFC. On the basis of its connectivity, the orbital and medial PFC was divided into orbital (OPFC) and medial (MPFC) 'networks' (Price, ANYAS, 2007, 1121:54-71). Injections of retrograde tracers in PFC produced dense to sparse labeling in the LC core. The distribution of this labeling varied with the location of the injection site, supporting the prior immunohistochemical evidence. In the MPFC network, injections in areas 24, 25, 32, 10, 14c, and the intermediate agranular insula (Iai) produced a moderate to dense labeling in LC, with injections in areas 24, 25 and 32 producing the densest labeling, and with injection in area 10m

producing more labeling than injection in area 10o. In the OPFC network, injections in area 13b, 12l, and the posterior median agranular insula (Iapm) produced dense labeling in LC whereas injections in area 11l produced only sparse labeling. The distribution of retrograde labeling in LC revealed a conspicuous overlap of cells labeled from distinct areas, with no obvious internal topography. Despite this conspicuous overlap, no double labeled cells could be observed in cases with injections of differently-colored tracers in distinct areas. This absence of co-localization is consistent with similar recent evidence obtained in rats (Chandler & Waterhouse, Front Behav Neurosci, 2012, 6:1-9) and suggests a complex spatial segregation of LC projecting neurons. The injection of anterograde tracers in PFC produced dense to sparse labeling predominantly in the direct periphery of the LC core. The examination of the distribution of this labeling indicated that the connections between LC and the different areas of OPFC and MPFC are rather reciprocal. In the MPFC network, injections in areas 24, 25, 32, 11m and Iai produced dense labeling whereas injections in 10m and 10o produced moderate and no labeling, respectively. In the OPFC network, injections in area 13l, Iapm, and the medial agranular insula (Iam) produced dense labeling whereas injections in areas 11l produced sparse labeling.

Disclosures: **M. Ubero Martinez:** None. **D. Hernandez:** None. **J.L. Price:** None. **R. Insausti:** None. **N.K. Logothetis:** None. **H.C. Evrard:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: HHS, NIH, NIMH IRP

Title: Afferent connections of the claustrum after injection of retrograde tracers in macaques (macaca mulatta)

Authors: M. GORSICH[1], R. GATTASS[2], M. MISHKIN[1,2], *R. C. SAUNDERS[1]
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Abstract: The claustrum is a thin, sub-cortical structure located between the insula and putamen. While it has been a focus of study for over 50 years little is known about its connectivity and function, particularly in primates. Purported functions range from a region important for

multisensory integration to the neural substrate for consciousness (Crick and Koch, 2005). Much of what we know about its anatomical connections has been gleaned through tracer injections into various cortical areas. To better understand the claustrum's functional role we examined its afferent connections by injecting anatomical tracers directly into the claustrum. Retrograde tracers (Fast blue, Flouro emerald, and Cholera toxin b) were injected bilaterally into two rhesus macaques with previous forebrain comissurotomies. Injection sites were placed at varying rostral-caudal (RC) and dorsal-ventral (DV) locations. There was widespread labeling throughout most of the neocortex, particularly in the frontal, temporal and occipital lobes. Within the frontal cortex the most consistent label from all injections was found in the orbital areas. Mid DV and RC injections also included label on the lateral surface, however only the mid DV injection resulted in label in the ventral medial frontal cortex. In the cingulate cortex the most ventral claustrum injection resulted in label in the more rostral area 24, while the mid-level injection's label was found throughout the rostral caudal extent of the cingulate. In the temporal lobe there was dense labeling throughout the rhinal cortex and, in some cases, in the posterior parahippocampal cortices of the medial temporal region. The inferior and superior temporal gyri, including the insular region, (Ig, Id, Ia, RT, and SII) were consistently labeled. Retrograde label was found in parietal areas 5 and 7 after mid DV and RC injections, but less so after rostral-ventral injections. Mid level injections resulted in label in the visual area V4, with more rostral injections resulting in label in V1. This study provides evidence that the primate claustrum receives input from both high level association areas such as the frontal cortex, as well as primary sensory areas such as VI and the auditory core.

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Poster

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Title: AMPA receptor GluR2 subunit expression level is upregulated in synapses of the cerebral cortex across primates

Authors: *T. I. DUKA[1], J. BAKER[1], Z. COLLINS[1], S. M. ANDERSON[1], M. RAGHANTI[2], J. J. ELY[3], P. R. HOF[4], D. E. WILDMAN[5], L. I. GROSSMAN[5], C. C. SHERWOOD[1]
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Abstract: Metabolic strategy and accompanying synaptic reorganization differ significantly among species. Our previous results indicate that there is differential metabolism in neuronal synaptic terminals that evolved among different primate lineages to meet the energy requirements at the subcellular level in neocortical cells. Therefore these metabolic changes might be associated with modifications in the molecular composition in post-synaptic specialization for elevated neuronal activity and plasticity. Also, because the prefrontal cortex is highly associated with the control of cognition and in the course of evolution, undergoes more expansion than rest of the brain, we aimed to examine the hypothesis that an acceleration of changes in the molecular framework of the PSD, in particular ionotropic glutamate receptor complexes, were associated with the evolution of prefrontal cortex in primates. We examined the expression level of NMDA receptor subunits (NR1, NR2A/B) and the AMPA receptor subunit (GluR2) in the neocortex and striatum in primates using quantitative Western blotting analysis of synaptosomal fractions. Quantitative Western blot analysis demonstrated that the expression of the GluR2 subunit of the AMPA receptor in the prefrontal cortex of haplorhines (i.e., monkeys, apes, and humans) was elevated by 42% as compared to strepsirrhines (i.e., lemurs and lorises), being most pronounced in chimpanzees and humans (70% greater than in strepsirrhines). In the striatum there were no significant differences in synaptosomal expression of ionotropic glutamate receptor subunits protein levels between groups. Our findings show that additional changes in glutamate metabolism took place in prefrontal cortex on the human evolutionary lineage.

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Poster

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Title: Marmoset motor cortex: Cortical input to areas 4 and 6D

Authors: *S. BAKOLA, K. J. BURMAN, M. G. P. ROSA
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Abstract: We describe topographic and quantitative aspects of connections to the primary motor (BA4, M1) and dorsal premotor cortex in the marmoset monkey (*Callithrix jacchus*), a small lissencephalic simian primate. Fluorescent retrograde tracers were injected in medial, intermediate, and lateral parts of M1, corresponding respectively to the representations of hindlimb/axial, forelimb, and head musculature, and in caudal (6DC) and rostral (6DR) premotor cortex. Marmoset M1 receives extensive input from premotor (6M, 6DC), somatosensory (3a, 3b, 1/2, S2), posterior parietal (PE, PF/PFG), and cingulate areas. The cingulate cortex targets preferentially medial M1; area 6DC, part of a wider cortical network for reaching, is strongly connected with intermediate M1. Lateral M1 has substantial connections with ventral premotor areas involved in grasping and face/mouth movements. Both premotor areas receive dense projections from medial premotor cortex. Area 6DC receives additional dense input from primary motor cortex and posterior parietal area PE. Other input originates in somatosensory areas S2, 1/2, 3a, and the anterior cingulate region. By comparison, area 6DR receives substantial input from prefrontal areas, mainly the area 8 complex, from the posterior cingulate and adjacent medial wall areas, and from visual-association parietal areas. Finally, dorsal premotor areas receive distinct input from ventral parietal cortex: 6DC from rostral areas PF/PFG, whereas 6DR preferentially from caudal areas PG/OPt. Marmoset motor and dorsal premotor connections are comparable to those reported for Old World monkeys, suggesting that

networks subserving sensorimotor transformations were established early in primate evolution. Thus, similar to what has been reported for macaques, the connections of M1 and 6DC emphasize a motor role for these areas; area 6DR is likely a site of convergence of highly-processed visual information with cognitive input, and can influence motor preparation via connections with caudal premotor areas. However, compared with macaques, our results suggest some differences in cingulate and ventral premotor projections to primary motor cortex, possibly reflecting the lack of precision grasping and the arboreal lifestyle of marmosets. Likewise, although aspects of parietofrontal organization parallel those reported in macaques, the relative paucity of 6DC connections with the putative homologues of macaque intraparietal areas is particularly surprising. Whether these discrepancies reveal genuine species differences, or are related to our current limited understanding of marmoset cortex remains to be addressed in future studies.

Disclosures: **S. Bakola:** None. **K.J. Burman:** None. **M.G.P. Rosa:** None.

Poster

446. Comparative Anatomy and Evolution I

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Topic: D.19. Comparative Anatomy and Evolution

Support: G. Harold and Leila Y Mathers Foundation

Title: Differences and similarities in neuron and cell packing densities across the cortical sheet in prosimian galagos and macaque monkeys

Authors: *E. C. ROCKOFF[1], N. A. YOUNG[2], D. K. FLAHERTY[1], J. H. KAAS[1]
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Abstract: According to previous research, cell and neuron densities vary across neocortex in a similar manner across primate taxa. Here, we provide a more detailed examination of this effect in two different primate taxa. We separated neocortex from the underlying white matter in 4 prosimian galago hemispheres (*Otolemur garnetti)*, and 4 macaque monkey hemispheres (*Macaca mulatta, Macaca nemestrina,* and *Macaca radiata*). The cortex was flattened into a sheet and cut into a number of small rectangles measuring approximately 4mm by 4mm. The number of cells and neurons were determined for each rectangle across the cortical sheet with flow cytometry (i.e., the flow fractionator method; Young et al., 2012; Collins et al., 2010). In brief, cells were labeled with fluorescent marker DAPI (4',6-diamidino-2-phenylindole) while neurons were immunolabeled for neuronal nuclear antigen (NeuN) that is present in neuronal

nuclei. In both primate species examined, primary visual cortex had the most densely packed neurons and primary motor cortex had the least densely packed neurons. Overall, neurons were more densely packed in a gradient from the rostral to the caudal pole. Neurons appear to be more densely packed in macaque neocortex than galagos. With some variability, results were similar across individuals within each species. The overall neuron densities across the whole cortex in galagos ranged from 6.2 to 12 million neurons/cm^2, while the overall neuron densities in macaques ranged from 9.3 to 20.3 million neurons/cm^2. The visual cortex neuron densities were predictably higher, ranging from 14.4 to 19.3 million neurons/cm^2 in galagos, and from 17.2 to 37.5 million neurons/cm^2 in macaques. The results extend and support other evidence that neuron packing densities vary across the cortical sheet in a predictable pattern within and across primate taxa.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: INSERM

MINISTERE AFFAIRES ETRANGERES - FRANCE

Title: The orbitofrontal cortex: Higher density of calretinin interneurons in the rhesus monkey than in the rat

Authors: D. DZAJA[1,2], Z. PETANJEK[2], *M. T. ESCLAPEZ[1,3]
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Abstract: Cortical column may have structural differences between species of different cognitive abilities. Several studies suggest that the ratio of excitatory versus inhibitory neurons, in cortex of different species, is not constant. This variability could result from evolutionary increased number of GABAergic neurons, in particular those containing calretinin (CR). In order to test this hypothesis, a quantitative study of the CR neuron density was performed in the orbitofrontal cortex (OFC) of wistar rats (n=3) and rhesus monkeys (n=2). The analyzed region was the medial orbital (MO) area in the rat and the Brodmann area 14 caudal (14c) in the

monkey. These two regions of the orbitofrontal cortex in the rat and monkey are known to receive strong projections from the hippocampal formation. In rat as in monkey, cresyl violet staining and immunohistochemical labeling for calretinin and NeuN were performed on adjacent sections throughout the entire region of interest. Unbiased stereological method was used to estimate the total number of neurons (NeuN) and CR cells in each cortical layer of the MO and 14c cortical area. The results demonstrate that the rat MO region, which displays a volume of 1.3mm^{3,} contain a mean total number of 114000 neurons (density: 86500/mm^3) and mean number of CR neurons of 3000 (density: 2200/mm^3). Therefore, CR neurons represent 2.6% of the total neuronal population in the MO area of the rat orbitofrontal cortex with the highest proportion of CR neurons observed in layer I (17% versus 3% for layer II; 3% for layer III; 1% in layer V, and 1% for layer VI). The monkey region 14c, which displays a volume of 13.8 mm^3, contains a mean total number of 1050000 neurons (density: 75800/mm^3) and mean number of CR neurons of 130000 (density: 9400/mm^3). Therefore, CR neurons represent 12% of the entire neuronal population in the 14c area of the monkey orbitofrontal cortex. The proportion of CR neurons per layer in 14c area is 55% in layer I, 15% in layer II&III, 6% in layer V and 5% in layer VI. This study establishes that the proportion of CR neurons is significantly higher in the rhesus monkey than in the rat orbitofrontal cortex.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: G. Harold and Leila Y. Mathers Foundation to JHK

Title: Cell number and volume of primary visual cortex in primates

Authors: *D. J. MILLER, R. PATHAK, P. BALARAM, J. KAAS
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Determining the cellular composition of specific brain regions is integral to our understanding of the function of the brain because it sheds light on the circuitry that makes up the neurobiological sensory systems that produce behavior. It is therefore useful to identify the extent to which the cellular composition of a single brain region varies across related taxa. In this

study, we estimated the volume and the number of cells in the primary visual cortex (area 17 or V1) of at least one species from each of the major subdivisions of the primate order using stereological procedures. Specifically, we used the optical fractionator to estimate the number of cells in V1 and the Cavalieri principle to estimate the volume of V1 from brain slices stained for Nissl substance. Our results indicate that the volume of V1 varies from approximately 0.2 mm3 to 7.8 mm3, and that the total number of cells in V1 ranges from approximately 80 million to 1.8 billion. Our data indicate that the density of cells in primate V1 range from approximately 200,000 cells/mm3 to over 500,000 cells/mm3. In addition, our results suggest that the number of cells in primate V1 scale as a linear function of V1 volume and that variation above or below this ratio between the size and the number of cells in V1 may be related to differences in behavioral ecology.

Disclosures: D.J. Miller: None. **R. Pathak:** None. **P. Balaram:** None. **J. Kaas:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Title: Morphology and topographical distribution of NADPH-diaphorase-labeled neurons and fibers in the human inferior colliculus

Authors: *L. EDELSTEIN[1], F. DENARO[2], D. HINOVA-PALOVA[3], B. LANDZHOV[3], M. MINKOV[4], L. MALINOVA[3], A. PALOFF[3], W. OVTSCHAROFF[3]
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Abstract: Using the NADPH-diaphorase histochemical technique, we investigated the morphology and topographical distribution of labeled neurons and fibers in the human inferior colliculus. NADPHd-positive neurons were distributed throughout its three major divisions: the central nucleus, the lateral nucleus and the pericentral nucleus. Relative to the other two divisions, large numbers of positive neurons were observed within the ventromedial aspect of the central nucleus. Taking into account the size and shape of labeled perikarya as well as their dendritic and axonal characteristics, neurons were categorized by diameter into three types: large, medium and small. Large neurons ranged from 30-45.5μm in diameter, their perikarya

representing a variety of shapes including elliptical, irregular, fusiform and multipolar. Medium neurons varied from 25-30µm in diameter, most often seen with multipolar, bipolar or irregular cell bodies. Small neurons ranged from 13-18µm in diameter and were typically oval or elliptical in shape. It was generally observed that the dendritic architecture of NADPHd-positive neurons consisted of both spiny and aspiny subtypes. As well, we demonstrated the existence of a dense neuropil of labeled fibers and fragments throughout the inferior colliculus, suggestive of the dendrites and axons of a robust network of NADPHd-positive neurons. Present results are supportive of our prior research in the cat (Paloff and Hinova-Palova, J. Hirnforsch., 1998, 39:231-243), and suggest the occurrence of two functionally distinct populations of NADPHd-positive neurons in the human inferior colliculus - [1] large and medium cells consistent with a projection neuron phenotype, and [2] small cells reflective of a local interneuron phenotype as defined by previous Golgi impregnation studies.

Disclosures: **L. Edelstein:** None. **F. Denaro:** None. **D. Hinova-Palova:** None. **B. Landzhov:** None. **L. Malinova:** None. **A. Paloff:** None. **W. Ovtscharoff:** None. **M. Minkov:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: DFG SU171

Title: Dendritic scaling in the cerebellar nuclei of rats and monkeys

Authors: ***F. R. SULTAN**[1], S. HAMODEH[1], A. BOZKURT[1], M. GLICKSTEIN[2]
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Abstract: Scaling of neurons has been proposed to conform to allometric laws determining the length of neuronal processes. Here we compared the dendritic length within a subcellular region, the deep cerebellar nuclei, in rats and monkeys in a systematic fashion. We find that the dendritic length density is remarkably constant within the two species and because the neuron density decreases, the dendritic length per neuron increases and would conform to an allometric scaling. Our analysis, however, also uncovers an important deviation from this allometric scaling in the dentate nucleus of the monkey. Here we observe a reduction in the dendritic length per neuron. A comparison to Golgi-stained neurons (Chan-Palay, 1977) shows that this is due to a smaller than expected dendritic fields that neurons have in the dentate nucleus of the monkey. These smaller dendritic fields in the dentate could lead to the folding observed in this nucleus in primates and

also could be functionally explained as being important for controlling sophisticated finger movements in primates. In summary, our findings show regular scaling of dendrites in the cerebellar nuclei and also point to deviations thereof in the phylogenetically newer nucleus that likely was important in shaping the primate brain.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: NIH Grant NS070717

Title: Biophysical constraints on the processing speed of axons conveyed by the corpus callosum: Possible role in the evolution of hemispheric asymmetry

Authors: *K. A. PHILLIPS[1,2], C. D. STIMPSON[3], J. B. SMAERS[4], M. RAGHANTI[5], A. POPRATILOFF[3], P. R. HOF[6], C. C. SHERWOOD[3]
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Abstract: Sending information through the nervous system is considerably more energetically costly for large brains than it is for small brains. This raises the possibility that inter-hemispheric communication may be constrained in larger brains as transmission delays increase. Two solutions for improving the rate of action potential conduction are to increase diameter of the axon and to myelinate the axon. However, there is not a uniform 'scaling up' of axon diameters and brain size, as it would lead to a prohibitively large brain due to increases in white matter mass. In particular, larger brains might be expected to have an increased proportion of larger diameter axons as a means to maintain conduction times. However, because of the significantly larger size of these axons, spatial packing constraints limit the rate at which these types of connections can increase. Determining characteristics of axon size distributions within the corpus callosum (CC) across primates can shed light on how these demands impact conduction speed and have been balanced in the evolution of the human brain. We studied phylogenetic variation

in axonal density and diameter of the CC in a sample consisting of brain specimens from 14 different anthropoid primate species, including New Word monkeys, Old World monkeys, apes, and humans. Axonal density and diameter were quantified using electron microscopy. The majority of myelinated axons were less than 1 micrometer in diameter across all species [median diameter = 0.56, SD = .13], indicating that conduction velocity for most inter-hemispheric communication is relatively constant regardless of brain size. Using PGLS to examine axon scaling relative to brain mass, we found that that scaling exponents for median axon diameter and density did not differ across the anteroposterior axis of the CC. However, the largest axons (those at the 95th percentile) scaled with a progressively higher exponent than the median axons towards the posterior region of the CC. The fastest cross-brain conduction velocities varied from < 3 ms to < 9 ms in the sample. Thus, even with an increase in axon diameter, inter-hemispheric transmission time is not maintained at a constant velocity with increasing brain size. Such biophysical constraints on the processing speed of axons conveyed by the CC may play an important role in the evolution of hemispheric asymmetry.

Disclosures: **K.A. Phillips:** None. **C.D. Stimpson:** None. **J.B. Smaers:** None. **M. Raghanti:** None. **A. Popratiloff:** None. **P.R. Hof:** None. **C.C. Sherwood:** None.

Poster

446. Comparative Anatomy and Evolution I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 446.24/MM28

Topic: D.19. Comparative Anatomy and Evolution

Support: University of Padova to BC, MG, SM, MP, AP

Title: The primary motor cortex of the horse. Comparison with other Perissodactyla and Primates

Authors: ***B. COZZI**[1], C. BALLARIN[1], C. BOMBARDI[2], P. CLAVENZANI[2], L. CORAIN[3], A. DE GIORGIO[4], M. GIURISATO[1], A. GRANDIS[2], S. MONTELLI[1], M. PANIN[1], A. PERUFFO[1], A. PIRONE[5], P. ZAMBENEDETTI[6], A. GRANATO[4]
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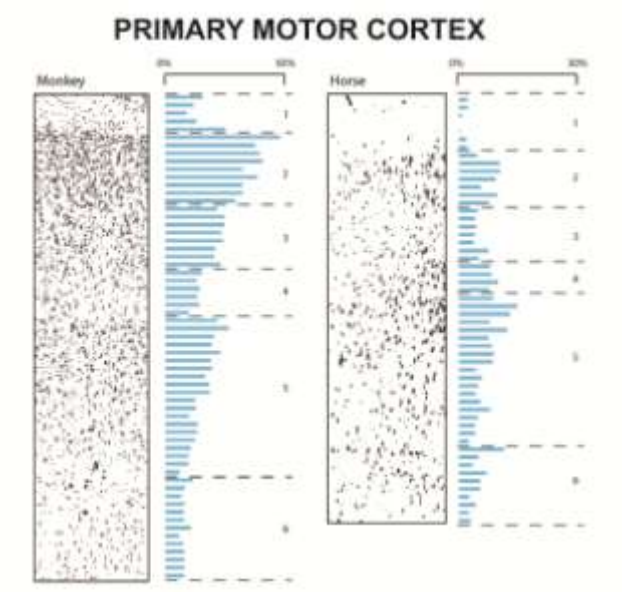
Abstract: The primary motor cortex (M1) is the region of the cerebral cortex influencing the integration of movement selection and initiation. It is well known that M1 combines inputs from other cortical motor areas, sensory areas and midbrain, and - in primates - its projections to spinal motoneurons are essential for fine control of the limbs. However there is very little information on the cytoarchitecture of the cerebral cortex of other mammals, including the even-toed Perissodactyla. In the present work we have analyzed the cortical stratification in the M1 of the horse and rhinoceros, and compared them to the corresponding areas of the crab-eating macaque, chimpanzee and man. Photomicrographs of Nissl-stained sections were thresholded with an image processing software, to obtain a relative quantification of cell populations within the cortical columns. Other sections were stained with peroxidase-immunohistochemistry to verify the distribution of selected calcium-binding proteins and specific neural markers. Our results show that the M1 of the horse shares many features with that of other ungulates (terrestrial Cetartiodactyla), with the notable exception of parvalbumin-positive cells that are widely spread across all layers, as observed in primates. The overall cortical mass was lower in the horse than in the macaque. Layer IV was very reduced or absent in the horse; a conspicuous layer I was present in the horse and rhinoceros, but very reduced in primates. Interestingly, the motor cortex of the horse showed a higher contribution of infragranular layers, whereas we observed a more relevant supragranular component in primates.



Disclosures: **B. Cozzi:** None. **C. Bombardi:** None. **P. Clavenzani:** None. **L. Corain:** None. **A. De Giorgio:** None. **M. Giurisato:** None. **A. Grandis:** None. **S. Montelli:** None. **M. Panin:** None. **A. Peruffo:** None. **A. Pirone:** None. **P. Zambenedetti:** None. **A. Granato:** None. **C. Ballarin:** None.

Poster

447. Comparative Anatomy and Evolution II

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.01/MM29

Topic: D.19. Comparative Anatomy and Evolution

Support: NSF Grant IOS 114661

Title: Family Matters: An intrafamily comparison of relative brain volumes within Mustelidae

Authors: *A. E. HRISTOVA[1], B. M. ARSZNOV[4], B. L. LUNDRIGAN[2], S. T. SAKAI[3]
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Abstract: Several comparative studies have examined the relationship between brain size and regional brain volumes in broad species comparisons across mammalian families. Multiple factors including sociality, diet, habitat, body size, and phylogeny have been found to correspond to variations in brain size. However, conflicting results have been reported from studies examining the same factors and brain size variations within single families. To explore this discrepancy, we examined interspecific variation in the brains of several species representing the largest family in the order Carnivora, family Mustelidae. This family contains an ecologically diverse set of species, ranging from the aquatic otters to burrowing badgers, and provides a unique opportunity to examine brain variation in some rarely studied carnivores. We used computed tomography to create three-dimensional virtual endocasts based on serial analysis of coronal sections through the adult endocranium (n=44) in 19 species, including representatives from both extant subfamilies, Lutrinae (7) and Mustelinae (12). A cluster analysis using ratios of regional brain volumes to the remaining endocranial volume revealed two main groups, which correspond to these subfamilies. Notably, ratios of total cerebrum and posterior cerebrum were significantly greater in Lutrinae than in Mustelinae, while ratios of olfactory bulb, anterior cerebrum, and cerebellum/brainstem were significantly greater in the Mustelinae than in Lutrinae. The brain variations observed between the two subfamilies are consistent with the influence of an aquatic versus a terrestrial environment on cognitive, spatial memory, and sensorimotor challenges. Interestingly, relative anterior cerebrum volume is greater in mustelines, particularly badgers and wolverines. However, whether ecological and behavioral variables influence variations in brain size within Lutrinae and Mustelinae requires further investigation. Nevertheless, these data support the notion that smaller scale comparisons may uncover morphological variation not detected by broader taxonomic comparative studies.

Disclosures: **A.E. Hristova:** None. **B.M. Arsznov:** None. **B.L. Lundrigan:** None. **S.T. Sakai:** None.

Poster

447. Comparative Anatomy and Evolution II

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Program#/Poster#: 447.02/MM30

Topic: D.19. Comparative Anatomy and Evolution

Support: NSERC

CFI

Title: Examination of sex differences in the volumes of brain and brain regions in the Eastern Chipmunk

Authors: R. DASENDRAN[1], G. SCOTT[1], H. LEHMANN[3], A. IWANIUK[4], *D. SAUCIER[2]
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Abstract: Many rodent species demonstrate size differences in body, brain and brain region volumes favouring males. The vast majority of these studies have focused on species with male-biased body size dimorphism,however, with little information available on sex differences in the brains of species with female-biased body size dimorphism. This study investigates sexual dimorphism in Eastern chipmunks, Tamias striatus, a rodent species with female-biased body size dimorphism. Based on their ecology and life history, we predicted that males would have larger hippocampal volumes than females because they are polygamous and males search over wide areas to find mates. We also tested for sex differences in brain regions that are often sexually dimorphic in species with male-biased body size dimorphism, namely, the prefrontal cortex and corpus callosum. Ten wild chipmunks were captured and euthanized at a number of field sites in Ontario from Apr. to Oct., a period of time that encompasses two breeding seasons. Brain region volumes were then measured with unbiased stereology from serial sections. Preliminary results confirm the body size advantage for females, but find limited support for corresponding volumetric differences in brain volumes or in specific brain regions. These results are consistent with Rensch's rule: sexual dimorphisms are smaller when females are the larger sex. Although these results are inconsistent with the prevailing theory that mate searching by

males of polygamous species is associated with a male bias in hippocampal volume, it does corroborate similar findings in other squirrel species. Further research is required to examine the effects of season and breeding status on these results.

Disclosures: **R. Dasendran:** None. **D. Saucier:** None. **H. Lehmann:** None. **G. Scott:** None. **A. Iwaniuk:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.03/MM31

Topic: D.19. Comparative Anatomy and Evolution

Support: NSF IOS-11212732

Title: Volume and cell density of midbrain regions in mice from lines selectively bred for high voluntary wheel running

Authors: ***Z. THOMPSON**[1], S. SHELTON[3], P. LEVIN[4], G. C. CLAGHORN[2], T. GARLAND, Jr.[2]
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Abstract: How does selection on a behavior alter brain structure? Mice that have been selectively bred for high voluntary wheel running show larger midbrains than their non-selected control counterparts (Kolb et al. 2013 J. Exp. Biol. 216:515-523). The midbrain contains regions involved in both reward and locomotor pathways. The goal of the current research is to determine which of these regions may have changed in the artificially selected mice. The Garland lab runs a selective breeding experiment that has been ongoing since 1993. Four replicate lines of high-runner (HR) mice are bred based on total number of wheel revolutions on days 5 & 6 of a 6-day period of wheel access. Four replicate control (C) lines are bred without regard to wheel running. HR mice voluntarily run ~3 times as much as C, primarily by running faster. 50 female mice were allowed access to wheels for 8 weeks (half HR and half C), while another 50 were kept without access to wheels (also half HR and half C). After the 8 weeks, all 100 mice were transcardially perfused, and the brains were dissected out and weighed. Brains were sliced at 40 microns on a cryostat, Nissl stained, and photographed. Regions of interest were traced and 3D volume reconstructions were made with Amira. Cell density was measured

with the ITCN plug-in for ImageJ. Nested ANCOVA in SAS Proc Mixed (with body mass as a covariate) showed a statistically significant ($P<0.05$) effect of both linetype (HR > C) and wheel access (access > no access) on brain mass, but no significant interaction. Preliminary analyses indicate no statistical differences in cell density in the substantia nigra or periaqueductal gray of the 50 mice that were not allowed wheel access. Analysis of the 50 mice that were allowed access to wheels is pending, and will allow us to determine if the larger midbrains of HR mice is a result of their increased running. In addition, other regions of the midbrain will be investigated, including the ventral tegmental area and raphe nucleus. We will also use this large dataset to examine other areas of the brain that we might expect to show effects of selective breeding and/or chronic exercise, including the hippocampus and corpus callosum.

Disclosures: Z. Thompson: None. **S. Shelton:** None. **P. Levin:** None. **G.C. Claghorn:** None. **T. Garland:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.04/MM32

Topic: D.19. Comparative Anatomy and Evolution

Title: A meta-analysis of multivariate analysis of brain mass correlations in eutherian mammals

Authors: *S. HUGGENBERGER[1,2], C. STEINHAUSEN[1,2], L. ZEHL[3], M. HAAS-RIOTH[4], K. MORCINEK[1], W. WALKOWIAK[2]
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Abstract: In this meta-analysis, a comprehensive sample of eutherian mammals (115 species distributed in 13 orders) provided data about several different biological traits and measures of brain size such as absolute brain mass (AB), relative brain mass [corrected by body mass (RB)], and encephalization quotient (EQ). These data were analyzed by multivariate statistics to show that brain size in general can be analyzed across orders independently from phylogenetic lineages. Species with high AB tend to (1) feed on protein-rich nutrition, (2) have a long lifespan, (3) delay sexual maturity, and (4) have long and rare pregnancies with small litter sizes. Animals with high RB usually have (1) a short life span, (2) reach sexual maturity early, and (3)

have short and frequent gestations. Moreover males of species with high RB also have few potential sexual partners. In contrast, animals with high EQs have (1) a high number of potential sexual partners, (2) delayed sexual maturity, and (3) rare gestations with small litter sizes. Based on these correlations, we conclude that Eutheria with either high AB or high EQ occupy high trophic levels. Eutheria of low trophic levels can develop a high RB only if they have small body masses.

Disclosures: S. Huggenberger: None. **C. Steinhausen:** None. **L. Zehl:** None. **M. Haas-Rioth:** None. **K. Morcinek:** None. **W. Walkowiak:** None.

Poster

447. Comparative Anatomy and Evolution II

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.05/MM33

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH-R01MH88

Title: Distribution of serotonergic markers in the brains of Japanese quail, European starlings, and zebra finches

Authors: *K. M. YODER, O. IYILIKCI, B. A. ALWARD, G. F. BALL
Johns Hopkins Univ., Baltimore, MD

Abstract: Serotonin has been implicated in a wide range of affective and cognitive processes. Recently it has been shown to modulate aspects of song production in songbirds and other functions in avian species. The present study aimed to examine the distribution of markers for the serotonin transporter (SERT) and the enzyme tryptophan hydroxylase (TPH) in the brains of 3 avian species that differ in song-learning and breeding type: European starling (open-ended learner, seasonal breeder), zebra finch (closed-ended learner, non-seasonal breeder), and quail (non-songbird). For all the species examined, dense TPH immunoreactive (ir) perikarya clusters were present in ventral and dorsal raphe at medulla oblongata; in the midbrain, TPH-ir cells were documented in the caudal linear nucleus, ventral tegmental area and a cluster of cells surrounded the medial longitudinal fasciculus. In the diencephalon, TPH-ir somata were present in the paraventricular nucleus and the premammillary nucleus. This system appears to be highly conserved across species, since these observations are consistent with what has been observed in other avian species and in mammals. SERT-ir fibers were widely distributed throughout the brain

in all 3 species examined, including the hypothalamus, nucleus accumbens, nucleus taeneia, periaqueductal grey and parts of hippocampus. SERT-ir fibers were observed in the rostral but not caudal regions of the bed nucleus of stria terminalis (BNST). Again, this pattern of distribution of SERT immunoreactivity is consistent with those previously reported in songbirds and in mammalian species. In addition, fiber tracts were observed along the retromammillary and dorsal premammillary nuclei. Within song control regions present in starlings and zebra finches but not quail, HVC and RA were sparsely labeled compared to surrounding nidopallium, whereas Area X had similar SERT staining as the surrounding striatum. Studies in the mammalian dentate gyrus of the hippocampus indicate that serotonin regulates neurogenesis. We are asking whether serotonin influences neuron incorporation in the song nucleus HVC in zebra finches by injecting the neurotoxin 5-7-DHT into the third ventricle and then measuring HVC volume and the number of cells immunolabeled with doublecortin (DCX), a marker of new neurons. Preliminary analyses have not detected a significant effect but the study is ongoing.

Disclosures: K.M. Yoder: None. **O. Iyilikci:** None. **B.A. Alward:** None. **G.F. Ball:** None.

Poster

447. Comparative Anatomy and Evolution II

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Program#/Poster#: 447.06/MM34

Topic: D.19. Comparative Anatomy and Evolution

Support: CONACyT Grant 167147

DGAPA-UNAM Grant IN213310

FES Iztacala UNAM Grant PAPCA-2009-2010

Title: Morphological and electrophysiological properties of spiny neurons in the striatum dorsolateral of the turtle

Authors: *J. BARRAL[1], C. GONZALEZ-SANDOVAL[1], J. MENDOZA SANCHEZ[1,2], M. MATA-HERRERA[3]
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Abstract: Comparative studies have shown similarities between reptilian and mammalian basal ganglia. Here the morphological and electrophysiological characteristics of the medium spiny

neurons (MSN) in the dorsolateral striatum (DLS) of the turtle are described after staining them with the Golgi techniques, or after intracellular recording, obtained from brain slice preparation. The somas of MSN in DLS were spherical, ovoid, or fusiform. The number of primary dendritic branches was less than observed in mammals. The origin of axon was similar to that observed in mammals. Dendritic spines were short, thin, bifurcated or fungiform; the length in dendritic spines exceeded that reported in mammals. MSN had similar to characteristics those reported in the mammalian, as observed in resting potential, delayed in the onset of the first action potential, rectification and adaptation in action potential firing at low frequencies. Differences observed could play an important role in the modulation of motor networks preserved along the vertebrate evolution.

Disclosures: J. Barral: None. **C. Gonzalez-Sandoval:** None. **J. Mendoza Sanchez:** None. **M. Mata-Herrera:** None.

Poster

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Support: NIH Grant DK081937 to AMK

NIH Grant GM109817 to AMK

UTEP Grand Challenges Award to AMK

NSF Chihuahuan Desert Biodiversity Summer REU Program

Title: Initial cytoarchitectonic characterization of the squamate forebrain: Case studies of the Western Diamondback rattlesnake (Crotalus atrox) and two distinct chameleon species (Trioceros jacksonii; Rieppeleon kerstenii)

Authors: D. F. HUGHES, K. PENNINGTON, E. M. WALKER, B. DE HARO, C. S. LIEB, E. GREENBAUM, *A. M. KHAN
Dept. of Biol. Sci. and Border Biomed. Res. Ctr., Univ. of Texas at El Paso, El Paso, TX

Abstract: The aims of this ongoing interdisciplinary team project at the University of Texas at El Paso are two-fold: (1) to begin characterizing the neural architecture of snakes occupying the arid regions of the Chihuahuan Desert region of West Texas as a basis for detailed examination of their behavioral adaptations to xeric environments; and (2) to begin characterizing the neural

architecture of chameleons originating in sub-Saharan Africa in order to identify soft tissue characters that would help clarify and inform ongoing phylogenetic analyses of these species by members of our team. To fulfill both of these distinct but related goals, we undertook a basic characterization of the gross neuroanatomy and cytoarchitecture of *Crotalus atrox* and *Trioceros jacksonii* / *Rieppeleon kerstenii*. An adult male *C. atrox* specimen was captured from the UTEP Indio Mountains Research Station and transported to the UTEP campus, where it was exanguinated under deep sedation. Similarly, the chameleon species (1 male and 1 female of *T. jacksonii*, and 1 female of *R. kerstenii*) were obtained from suppliers in Hawaii and transcardially perfused with saline followed by 4% paraformaldehyde (PFA) in solution. In all cases, brains were cryoprotected in sucrose/PFA and then frozen in hexane. They were cut into 20 μm-thick sections and collected into four tissue series. The first series of the forebrain was Nissl-stained to ascertain the cytoarchitecture of each tissue section. The Nissl series from the rattlesnake was aligned with previous cytoarchitectonic data published for the related species *C. viridis*, and tissue series are now being immunoreacted for neuropeptide staining. For the chameleon brains, the gross neuroanatomy revealed a marked specialization of the brain for visual processing, with unusually developed optic chiasm and overlying diencephalon. The basic gross neuroanatomy of *R. kerstenii*, the pygmy chameleon, was markedly different from *T. Jacksonii* in terms of overall shape and appearance of major divisions of the brain, the structure of the lobes and surface anatomy, and the relative proportions of tissue devoted to major subdivisions. Further analysis of differences in major cytoarchitectural features, including organization of neuronal populations into regions and nuclei, is currently underway. Our data provide the first views of the brains for these species of chameleon, and together with the rattlesnake brain tissue we have examined, provide a useful starting framework for further neuroanatomical comparisons and characterizations to be made.

Disclosures: D.F. Hughes: None. **A.M. Khan:** None. **K. Pennington:** None. **E.M. Walker:** None. **B. De Haro:** None. **C.S. Lieb:** None. **E. Greenbaum:** None.

Poster

447. Comparative Anatomy and Evolution II

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Program#/Poster#: 447.08/MM36

Topic: D.19. Comparative Anatomy and Evolution

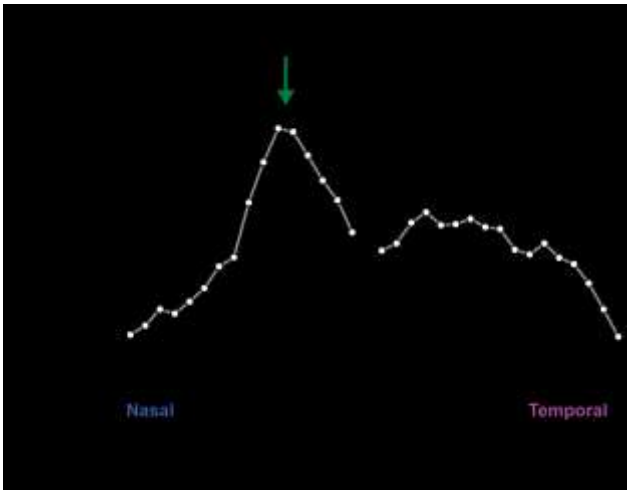
Support: Pew Charitable Trusts

NEI Grant

Title: A high density region of retinal ganglion cells in the grasshopper mouse

Authors: *B. SCHOLL, T. A. CLARK, N. J. PRIEBE
Section of Neurobio., The Univ. of Texas At Austin, Austin, TX

Abstract: Along the mammalian phylogeny there exist differences in the anatomy and physiology of animals exhibiting predatory or foraging behavior. In particular, features of retina show marked differences between species in the orders carnivora and rodentia. Many rodents, having laterally-shifted eyes and a modest binocular field of view, show a relatively uniform sampling across the retina in terms of ganglion cell density. In contrast, carnivorans such as cats have a large binocular field of view and retinas containing a specialized region of high ganglion cell density, known as the area centralis. We examined whether a predatory rodent, the grasshopper mouse (Onychomys arenicola), has a different ganglion cell distribution from the C57/BL6 lab mouse, an herbivore. Retinas from grasshopper mice (n = 3) and lab mice (n = 3) were enucleated, immersed in formalin, dissected, and stained with DAPI for cellular visualization using confocal microscopy. Images (150 x 150 microns) were collected along both naso-temporal and dorsal-ventral axes. The average retinal ganglion cell density in the grasshopper mouse was 5518 cells/mm2, markedly lower than that of the C57/BL6 mouse which had an average density of 7981 cells/mm2. Furthermore, regardless of location along the central meridian, ganglion cell densities at the furthest eccentricities were sparser in the grasshopper mouse compared to the C57/BL6 mouse. Strikingly, there was a distinct asymmetry along the naso-temporal retinal axis in grasshopper mice evident by a large increase in ganglion cell density near the optic tract. Along the nasal axis, at eccentricities close the optic tract (<1.5mm), average ganglion cell density was higher (7576 ± 434 cells/mm2, mean ± s.e.) than those farther away (>1.5mm; 3918 ± 423 cells/mm2, mean ± s.e.). This feature was also evident along the dorsal-ventral axis. These observations suggest that, unlike widely-used C57/BL6 lab mice, predatory grasshopper mice have developed a specialized region of their retina to convey visual information with higher spatial resolution.



Disclosures: **B. Scholl:** None. **T.A. Clark:** None. **N.J. Priebe:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.09/NN1

Topic: D.19. Comparative Anatomy and Evolution

Support: Grant 14/2013 of the Medical University of Sofia, Bulgaria

Title: Alteration of CB1 receptor density in the amygdala by kyotorphin

Authors: L. EDELSTEIN[1], *F. J. DENARO[2], B. LANDZHOV[3], E. DZHAMBAZOVA[4], L. MALINOVA[3], D. HINOVA-PALOVA[3], A. PALOFF[3], W. OVTSCHAROFF[3]
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Abstract: There is considerable evidence describing the commonality of opioid and cannabinoid receptor systems. Opioid peptides are widely known as modulators of numerous CNS functions, in particular, nociception. In addition to abundant extant literature on this topic, data from our previous investigations show that the short-chain neuropeptide and neuromodulator kyotorphin (KTP) binds to a specific receptor and plays a role in pain regulation, thermoregulation and exploratory behavior (Dzhambazova and Bocheva, J. Biomed. Clin. Res., 2010, 3:3-11;

Dzhambazova et al., Amino Acids, 2011, 4:937-944). According to several studies, the action of KTP on integrative brain functions in animals is not blocked by the pure opioid antagonist naloxone. This fact has led to much investigation of its interaction with monoaminergic systems. However, to our knowledge, the interaction between KTP and cannabinoid systems has yet to be studied. The aim of the present study was to investigate the effect of KTP on the regulation of CB1-immunopositive neurons in the amygdala of the male Wistar rat using AM251, a cannabinoid receptor antagonist (inverse agonist). Results of the present research revealed that the density of CB1-positive neurons, processes and neuropil throughout the amygdala was significantly greater in the KTP-treated subjects than in controls. Morphometric analysis revealed a 40% increase in receptor density. This effect was completely blocked by the administration of AM251, clearly indicating that KTP interacts with CB1 receptors. In all likelihood, both monoaminergic and opioidergic mechanisms are directly influencing the functionality of endocannabinoid neurons. The involvement of KTP in the endocannabinoid signaling system is further evidence for its essential role in the regulation of animal behavior.

Disclosures: L. Edelstein: None. **F.J. Denaro:** None. **B. Landzhov:** None. **E. Dzhambazova:** None. **L. Malinova:** None. **D. Hinova-Palova:** None. **A. Paloff:** None. **W. Ovtscharoff:** None.

Poster

447. Comparative Anatomy and Evolution II

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.10/NN2

Topic: D.19. Comparative Anatomy and Evolution

Support: NSERC G121210071

Title: The retinal projection to the pretectal nucleus lentiformis mesencephali in pigeons

Authors: *D. WYLIE[1], J. KOLOMINSKY[2], D. J. GRAHAM[2], T. J. L:ISNEY[2], C. GUTIERREZ-IBANEZ[2]
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Abstract: In birds, the nucleus of the basal optic root (nBOR) and the nucleus lentiformis mesencephali (LM) are retinal-recipient nuclei involved in the analysis of optic flow and the generation of the optokinetic response to facilitate retinal image stabilization. The nBOR receives retinal input largely, if not exclusively, from displaced ganglion cells (DGCs), which are found at the margin of the inner nuclear layer and inner plexiform layer, rather than the ganglion

cell layer. The LM receives afferents from retinal ganglion cells (RGCs), but whether DGCs also project to LM remains unclear. To resolve this issue, we made small injections of retrograde tracer into LM and examined horizontal sections through the retina. For comparison, we also had cases with injections in nBOR, the optic tectum (TeO) and the anterior dorsolateral thalamus (DLL) (the equivalent to the mammalian lateral geniculate nucleus). From all LM injections both RGCs and DGCs were labelled. The proportion of DGCs varied from 2-28%, and these were not different in morphology or size compared to those labelled from nBOR (diameters; LM = 21.2 ± 3.42µm, nBOR = 22.7± 3.84µm (mean ± s.d.)). The proportion of DGCs labelled from the nBOR injections was much higher (84-93%). DGCs were also labeled after injections into the DLL. The proportion was small (2-3%), and these DGCs were smaller in size (diameter = 16.6 ± 1.86µm) than those projecting to the nBOR and LM. There were some obvious differences with respect to the sizes of the RGCs labelled from injections in LM, nBOR, TeO and DLL. Overall, The diameter of the RGCs varied from 5 to 25µm. Those labelled from TeO were the smallest and showed the least variability (diameter = 6.81 ± 0.9µm). The LM-projecting RGCs were bigger on average (diameter = 10.61 ± 2.84µm), but showed more variability. While most (49%) were as small as the TeO-projecting RGCs (5-10µm), there were many LM-projecting RGCs with diameters in the 10-15µm range, and some even larger RGCs approaching the size of the DGCs . The DLL-projecting RGCs were even larger, averaging 14.42 ± 2.70µm in diameter. The RGCs labelled from the nBOR injections were the largest (diameter = 16.21 ± 3.58µm). Although a few small nBOR=projecting RGCs were observed, most (48%) were in the 10-15µm range. 17% of the nBOR-projecting RGCs were as large as the DGCs (20-25µm range). Based on this analysis of size, we suggest that different populations of RGCs and DGCs are involved in the projections to LM, nBOR, TeO and DLL.

Disclosures: D. Wylie: None. **J. Kolominsky:** None. **D.J. Graham:** None. **T.J. L:isney:** None. **C. Gutierrez-Ibanez:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.11/NN3

Topic: D.19. Comparative Anatomy and Evolution

Title: A computational model of the octopus arm network supports modular distributed processing of sensory information

Authors: *F. W. GRASSO
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Abstract: Octopus are invertebrates with advanced nervous systems that support advanced learning capabilities and sophisticated behavioral repertoires that are comparable to those of mammals. The architecture of the cephalopd CNS is fundamentally different from that of vertebrates having diverged from their common ancestor at least 504 million years ago. One architectural difference between the vertebrate and the cephalopd CNS is that 2/5 of the 10 8 cephalopd CNS are bilaterally organized, in the cerebral ganglion and 3/5ths form a modular distributed network of ganglia amongst the 8 arms and the brachial ganglia that connects those eight arms. The complex behavioral repertoires of the octopus are thought to emerge from programs the distributed arm ganglia mediated by descending executive control from the bilaterally-organized cerebral ganglia. In simulation studies we modeled chains of sucker and brachial ganglia with 21 interconnected arm-section models to study the dynamics of information transmission within the octopus arm. Systematic variation of the range of inter-module connectivity, paralleling the variation of anatomical connectivity differences provided different dynamic regimes and durations of information persistence in the network. This homogeneity of structure may support the simultaneous existence of alternative representations in the octopus arm.

Disclosures: F.W. Grasso: None.

Poster

447. Comparative Anatomy and Evolution II

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Topic: D.19. Comparative Anatomy and Evolution

Support: Programa Bicentenario Becas Chile Scholarship to C. A. Salas

Australian Research Council Discovery Grant DP120102327 to S. P. Collin

Title: Terminal nerve-like receptors in the main olfactory epithelium of the precocious lamprey Mordacia praecox

Authors: *C. A. SALAS[1], N. S. HART[1], H. S. GILL[2], I. C. POTTER[2], S. P. COLLIN[1]
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Abstract: The presence of a terminal nerve complex (TN) in lampreys, including an olfacto-retinalis component, has previously been the subject of much debate. Although extra-bulbar olfactory projections (EBOP) with similar characteristics to the TN found in gnathostomes have been described in lampreys, the absence of a ganglion and the lack of both gonadotropin-releasing hormone (GnRH)- and FMRFamide peptide immunoreactivity (ir) suggest that lampreys may not possess a TN, where TN is an innovation of gnathostomes. Here, we describe EBOP in *Mordacia praecox* that may serve a comparable function to that of the TN of gnathostomes. Trans-synaptic labeling of ciliated receptors was observed in the main olfactory epithelium (MOE) after an intraocular application of the beta subunit of Cholera toxin (CTb) in adult specimens of *M. praecox*. Labeled cells were arranged as a pseudo-stratified epithelium and distributed in patches of irregular density across the MOE. Labeled receptors were found to project collateral fibers to cells located in the epithelium of the accessory olfactory system, where terminals were mostly confined to an area surrounding the cell bodies. These MOE fibers then joined the olfactory nerve, entered the olfactory bulb (OB) medially and decussated into two fascicles; one dorsally oriented that terminated in heavily stained varicosities in the dorsomedial neuropil in the posterior OB, and the other ventrally oriented that reached the septal nucleus before descending ventrally, rostral to the optic chiasm in the preoptic area (PA). We also found fibers in the striatum, the PA, the dorsal and ventral hypothalamus (Hyp), the nucleus of the posterior commissure (NPO), and the posterior tuberculum. However, due to our experimental approach, we cannot rule out that other afferent fibers innervate these areas. As previously described in other species of lampreys, we could not find a retinopetal ganglion of the TN or any GnRH-ir EBOP receptors in *M. praecox*. We infer that the trans-synaptic transport of CTb in the EBOP occurs from synapses located along the PA-Hyp-NPO axis, which is the first region of integration of retinal axons. This region contains FMRFamide-ir retinopetal cells and most of the GnRH-ir cells described in the brain of other lampreys. If this was the case in *M. praecox*, then the EBOP could exert TN-like control of the reproductive function through the direct modulation of their activity. Similarly, EBOP terminals surrounding somata in the accessory olfactory epithelium and along their projections suggest that EBOP receptors participate in pheromone perception and mating in lampreys.

Disclosures: C.A. Salas: None. **N.S. Hart:** None. **H.S. Gill:** None. **I.C. Potter:** None. **S.P. Collin:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

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Program#/Poster#: 447.13/NN5

Topic: D.19. Comparative Anatomy and Evolution

Support: CURAS Faculty Research Fund

Title: Olfactory and vomeronasal systems are both present in a cartilaginous Holocephalian elephant shark, Callorhinchus milii

Authors: *L. L. BRUCE[1], K. J. QUANDT[1], M. L. BARTLETT[2], E. GARZA-GISHOLT[3], S. P. COLLIN[3]
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Abstract: The olfactory epithelia of teleosts and tetrapods contains different types of olfactory sensory neurons (OSNs) that express a single functional odorant receptor protein and project to a glomerulus, which is innervated by only one or a few types of OSNs and are segregated into predominantly olfactory and vomeronasal regions. These project to distinct forebrain targets, i.e. the main and accessory olfactory bulbs (MOB and AOB) in lungfishes and tetrapods, but project to selective glomeruli within the olfactory bulb in teleosts. Holocephalians (chimaeras) are a subclass of cartilaginous fishes that are derived from the common ancestor of jawed vertebrates, and thus are a critical reference for understanding vertebrate evolution. This study tests the hypothesis that olfactory and vomeronasal OSNs occupy distinct regions of the olfactory epithelium and project to distinct forebrain targets in the Holocephalian elephant shark, *Callorhinchus milii*. In *C milii*, the olfactory epithelium contains distinct OSN types, which differ in location, morphology, and antibody labeling characteristics. Anti-$G\alpha_{s/olf}$ was used to identify olfactory neurons, anti-$G\alpha_o$ and anti-calbindin were used to identify vomeronasal OSNs, and anti-calretinin was used to identify a combined population. Both anti-calretinin and anti-$G\alpha_{s/olf}$ label tall ciliated OSNs located throughout the olfactory epithelia. Anti-$G\alpha_o$ predominantly labels neurons with short dendritic protrusions (called crypt cells) located close to the midline raphe of each olfactory epithelium, and is known to label vomeronasal OSNs in other vertebrates. Anti-calbindin labels a subset of the anti-calretinin immunoreactive tall ciliated OSNs, which are located close to the midline raphe of the olfactory epithelia. In the olfactory bulb, anti-tyrosine hydroxylase intensely labels neurons and axons throughout both the main and accessory bulbs, and distinguishes them from the rest of the forebrain. Anti-calretinin and NADPHd label terminals in the glomerular layer of both the MOB and AOB. Anti-calbindin labels terminals in the glomerular layer of the AOB. Calbindin is a marker for the AOB in lungfishes and tetrapods. Thus, the MOB and AOB can be identified in *C milii* using the same characteristics used to identify them in lungfishes and tetrapods. These results demonstrate that in *C milii*, both olfactory and vomeronasal OSNs are present in the olfactory epithelium, with a

vomeronasal region located near the midline raphe. Furthermore, the forebrain contains distinct olfactory and vomeronasal targets, suggesting that distinct olfactory and vomeronasal systems were present in the common ancestor of jawed vertebrates.

Disclosures: L.L. Bruce: None. **K.J. Quandt:** None. **M.L. Bartlett:** None. **E. Garza-Gisholt:** None. **S.P. Collin:** None.

Poster

447. Comparative Anatomy and Evolution II

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 447.14/NN6

Topic: D.19. Comparative Anatomy and Evolution

Support: Department of Biology, UNCG

Title: GABAergic neurons occur in larvae of the marine snail Ilyanassa obsoleta

Authors: P. A. DHARMASRI, C. S. GUNN, *E. M. LEISE
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Abstract: Several neurotransmitters, including nitric oxide and serotonin, regulate metamorphosis in marine molluscs. Our recent evidence suggests that γ-aminobutyric acid (GABA) is also active in the inhibitory regulation of this crucial developmental event (Biscocho, 2013). During metamorphosis, as the organism transitions from a planktonic, larval existence to a benthic, adult form, snails like Ilyanassa obsoleta lose their swimming and feeding organs, the velar lobes, and an anterior brain ganglion, the apical ganglion (AG), also known as the apical sensory organ. The AG is known to respond to metamorphic inducers in related molluscs and controls velar activities. We are using immunocytochemistry (ICC) to identify and localize GABAergic neurons in larval snails. Results demonstrate GABA-like immunoreactive fibers in the velar lobes and multiple cells in the pedal ganglia. GABAergic immunoreactivity also occurs in some specimens in the AG. In addition to the inhibition of metamorphosis (Biscocho, 2013), GABA may be involved in the regulation of swimming behaviors. Future studies will follow changes in GABAergic populations through development and metamorphosis using both ICC and CLARITY-inspired method (Chung et al., 2013).

Disclosures: P.A. Dharmasri: None. **C.S. Gunn:** None. **E.M. Leise:** None.

Poster

447. Comparative Anatomy and Evolution II

Location: Halls A-C

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Program#/Poster#: 447.15/NN7

Topic: D.19. Comparative Anatomy and Evolution

Support: University of Cologne

Title: The characterization of the vocal pathway of the fire-bellied toad, bombina orientalis

Authors: *S. MAIER[1], W. WALKOWIAK[2]
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Abstract: In anuran amphibians, the pretrigeminal nucleus, also known as dorsal tegmental area of the medulla (DTAM), is a crucial part of the vocal pattern generator for the production of advertisement calls. Its relation to vocalization was first established by the results of lesion and stimulation experiments. Whereas lesions eliminated all vocal behavior, electrical stimulation resulted in the production of well-patterned calls. A second semi-independent generator is postulated in the region of the motor nuclei IX-X/XII. The first anatomical studies in the aberrant species *Xenopus laevis*, using HRP-WGA and fluorescent amines, supported the hypothesis that the DTAM is involved in vocalization, because it projects into relevant motor areas. Besides these projections it has been shown that the DTAM is also connected reciprocally to the ventral striatum, the dorsal infundibular nucleus and the rostral raphe pars dorsalis. A direct projection from DTAM to the preoptic area, which was orignally assumed, could not be verified. The preoptic area, especially the anterior part, plays an important role in both reproduction and call production. It is regarded as the vocal pacemaker nucleus. It is still not known how the input from the anterior part of the preoptic area into the pretrigeminal nucleus area is organized. Our aim was to investigate the vocal pathway of the pedomorphic *Bombina orientalis*, with special emphasis on the role of afferent input to the praetrigeminal nucleus. As a first step the *c-fos* staining method, which marks activity-dependent immediate early gene expression, was used to map brain activity in calling males. Labeling of neurons within the vocal areas was compared between males that were exposed to conspecific calls and those that were not stimulated. Acoustic signals of conspecific advertisement calls lead to *c-fos* expression in neurons in the anterior part of the preoptic area, the dorsal infundibulum nucleus, the posteroventral tegmentum, and the pretrigeminal nucleus. The connectivity of vocal areas was analyzed with the marker biotin and fluorescent tracers. Tracer applications into the pretrigeminal nucleus (vocal pattern generator) revealed no direct projection from the anterior preoptic nucleus (vocal pacemaker), but instead from the tegmentum, infundibulum and the raphé nucleus. Trace

application into the preoptic area confirms that the pattern generator and vocal pacemaker are connected through relay stations, probably the infundibulum and the tegmentum. The tegmentum shows reciprocal connections to both vocalization areas and to the infundibulum, so that its role as part of an audio-vocal interface is evident.

Disclosures: S. Maier: None. **W. Walkowiak:** None.

Poster

447. Comparative Anatomy and Evolution II

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Topic: D.19. Comparative Anatomy and Evolution

Support: National Science Foundation Graduate Research Fellowship Grant DGE- 1143953, 7/15/2011-6/30/2016

Title: Genealogical correspondence of learning and memory centers across phyla

Authors: *G. H. WOLFF, N. J. STRAUSFELD
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Abstract: In most bilaterian animals, neurons from primary sensory neuropils project to higher order centers, such as the ascending relays from olfactory glomeruli to the mushroom bodies in arthropods and annelids, and from the olfactory lobes to the hemiellipsoid bodies in malacostracans. In mammals, the primary olfactory bulb projects interneuron relays to the hippocampus's dentate gyrus via the entorhinal cortex. In numerous species, such higher centers both integrate multimodal sensory inputs, and have properties demonstrating their roles in learning and memory, hence their description as "learning and memory" neuropils. In the present study, we have used immunohistochemistry and neuronal staining to localize and compare key characters of these centers including protein expression and structural morphology in taxa belonging to Insecta, Crustacea, Myriapoda, Chelicerata, Annelida, Platyhelminthes and Chordata. The aim has been to determine whether the mushroom body ground pattern genealogically corresponds across phyla and, if it does, whether this supports the proposition that the same ground pattern was present in the common ancestor of protostomes and deuterostomes. Despite taxonomic differences of forebrain morphologies, we have found PKA-Cα, 14-3-3ζ, and CaMKII expression preferentially localized to paired centers within the first brain segment. Furthermore, within each representative taxon of each major branch of the bilaterian

phylogenetic tree, we have identified unifying organizational principals pertaining to neuron arrangements in paired centers that are specially resolved by antisera raised against PKA-Cα, 14-3-3ζ, and CaMKII. These findings support the parsimonious view that the protostome-deuterostome ancestor was equipped with paired mushroom body-like sensory association centers, which as a requirement for exploratory behaviors detected and stored chemical information about its ecology. Evidence of such behaviors is today found in trace fossils that exist at the Ediacaran-Cambrian boundary.

Disclosures: G.H. Wolff: None. **N.J. Strausfeld:** None.

Poster

447. Comparative Anatomy and Evolution II

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Topic: D.19. Comparative Anatomy and Evolution

Support: Leverhulme Trust Research Project Grant (F/00 696/T)

Title: Neural ground patterns in deep time

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Abstract: The period of rapid evolutionary diversification known as the Cambrian explosion provides the first clear view of animals occupying a variety of definable ecological niches. In a sense, relationships amongst animals then, and the habitats they occupied, were not principally different from those in today's shallow seas and reefs where predatory euarthropods, such as mantis shrimps equipped with exquisite vision and grasping appendages, prey on smaller crustaceans and molluscs. Five hundred and twenty million years ago, as now, bilaterians crawled along the seabed, vermiform animals climbed and browsed on marine vegetation, and a variety of segmented arthropod-like animals walked, swam, and burrowed. Menacing this menagerie were the largest animals in the Cambrian seas, top predators known as Anomalocaridida or Radiodonta. Each was equipped with large compound eyes set on stalks flanking a cone-like mouth lined with many small teeth[1,2]. These animals differed from other segmented arthropods having jointed appendages or swimmerets in that they possessed just one pair of articulating appendages. Equipped with sharp spines, these extended preocularly from the front of the head, curving forwards and downwards ready to seize prey. Elsewhere along the

body, metameric arrangements of lateral flaps likely provided a metachronal wave that propelled the animal through the water. An anomalocaridid's smörgåsbord included animals distinct from, and much simpler than extant arthropods. Indeed, many of the taxa then did not obviously correspond to the living groups within phyla and for this reason are referred to as stem arthropods[3]. But how different were they? Were their behaviors driven by nervous systems fundamentally different from those existing today? Can we recognize features that suggest similarities of sensory integration, motor control and behavior? In this talk, I will demonstrate that already in the early Cambrian nervous systems existed that we can recognize as belonging to three major groups of arthropods alive today[4-6]. These nervous systems, preserved in 520-million-old Chengjiang fossils from southwest China, suggest that today's behavioral repertoires originated long before the evolution of morphologies that characterize modern taxa. 1. Daley AC, Edgecombe, GD. *J. Paleontol*. 88, 68-91, 2014. 2. Paterson JR et al. *Nature* 480, 237-240. 2011. 3. Budd GE, Jensen S. *Biol. Rev.Cambridge Phil. Soc*. 75, 253-295. 2000. 4. Ma X-Y, Hou X, Edgecombe GD, Strausfeld NJ. *Nature* 490, 258-261. 2012. 5. Tanaka G, Hou X-G, Ma X-Y, Edgecombe GD, Strausfeld NJ. *Nature* 502, 364-367. 2013. 6. Cong P, Ma, X-Y, Hou X-G, Edgecombe GD, Strausfeld NJ. submitted. 2014.

Disclosures: N.J. Strausfeld: None. **G.D. Edgecombe:** None. **X. Ma:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.01/NN10

Topic: E.01. Neuroendocrine Processes

Support: NSF IOS 0743818

Title: Functional evolution of gonadotropin-releasing hormone and adipokinetic hormone: Studies from the sea hare, *Aplysia* californica

Authors: *J. I. JOHNSON, P.-S. TSAI
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Abstract: In vertebrates, gonadotropin-releasing hormone (GnRH) is required for reproductive activation and hence the propagation of species. Emerging evidence suggests that GnRH and several related peptides, including adipokinetic hormone (AKH), are members of the GnRH superfamily that arose around 680 million years ago. Although the reproductive role of GnRH is

well established among vertebrates, such a role for other GnRH superfamily members remains controversial in protostomes. To gain insights into the biological function of protostomian GnRH homologs, we examined the expression pattern and biological activities of a GnRH (ap-GnRH) and AKH (ap-AKH) in a gastropod mollusk, *Aplysia californica*. To date, *A. californica* is the only known protostome that simultaneously expresses an endogenous GnRH and AKH and therefore represents an excellent model for examining the functional evolution of two related peptides in a single species. Localization of ap-AKH and ap-GnRH using *in situ* hybridization (ISH) and immunocytochemistry (ICC) revealed that they were produced in different regions of the central nervous system (CNS), with ap-GnRH produced by ganglia implicated in motor control, and ap-AKH by ganglia implicated in the homeostatic control of physiological functions. The *in vivo* effects of ap-GnRH and ap-AKH were consistent with their distribution patterns. Specifically, ap-GnRH injections triggered motor responses of the foot and parapodia, whereas ap-AKH injections triggered physiological responses such as reduced body mass and increased gut motility. Lastly, neither ap-AKH nor ap-GnRH acutely activated *A. californica* reproduction. These results support the hypothesis that the ancestral molecule that gave rise to GnRH and its homologs may be a more general neuroregulator responsible for diverse functions, and that reproductive activation may be a vertebrate-specific adaptation for GnRH signaling after the acquisition of the adenohypophysis.

Disclosures: J.I. Johnson: None. **P. Tsai:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

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UCSF Diabetes Family Fund

AHA Grant-in-Aid 13GRNT16120004

Title: Unraveling the molecular complexity of estrogen-responsive neurons in the ventromedial hypothalamus

Authors: *W. C. KRAUSE, H. A. INGRAHAM
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Abstract: The ventromedial nucleus of the hypothalamus (VMH) regulates a variety of physiological and behavioral functions, including those associated with reproduction, metabolic homeostasis, fear, and aggression. Several of these functions are sex-specific and involve hormone-dependent nuclear receptors. In particular the estrogen receptor alpha (ERα) is restricted to the ventrolateral subdivision of the VMH (VMHvl), and previous studies in rodents have demonstrated that ERα and/or the neurons expressing this receptor are necessary for the reproductive, homeostatic, and aggressive behaviors influenced by the VMH. Given the molecular and functional complexity of the VMH, we hypothesize that subpopulations of ERα-positive neurons with distinct molecular signatures help to generate the complex responses mediated by this neuroendocrine region. To molecularly define the VMH neurons that acutely respond to estradiol we are using phosphorylated ribosome capture (PRC). Neuronal stimulation often leads to phosphorylation of the S6 ribosomal subunit (pS6), and this modification can serve as a tag to immunoprecipitate RNA specifically from such activated neurons. Four hours after subcutaneous injection of estradiol benzoate (EB) to female mice with low levels of endogenous estrogens--either as a consequence of ovariectomy or estrus cycle stage--we observe a significant and robust increase in pS6 immunoreactivity (IR) in the region of the VMHvl that overlaps with ERα IR; many of these pS6-positive cells are also positive for ERα. No such increase is observed after treatment with vehicle alone. Further, using a conditional knockout strategy to eliminate ERα expression in the mediobasal hypothalamus, we find no induction of pS6 by EB, suggesting that the observed increase in pS6 IR is a cell autonomous response to hormone treatment. We are currently using RNA-Seq to profile the RNA enriched by PRC from these EB-responsive neurons in the hypothalami of wild type and ERα conditional knockout mice. Because pS6 appears to be a reliable maker of acute estrogenic activity in the hypothalamus, this method is also being used to explore the effects of age and sex on the mechanism by which ERα mediates this activation.

Disclosures: W.C. Krause: None. **H.A. Ingraham:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R01 MH057759

NIH Grant 5T32GM008328-22

Title: Neural growth hormone: Regional regulation by estradiol and/or sex chromosome complement

Authors: ***E. HARRIS**, K. QUINNIES, P. BONTHUIS, E. RISSMAN
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Abstract: Growth Hormone (GH) is a large peptide hormone synthesized in the pituitary gland and brain. In the hippocampus sex differences are caused by estradiol, but in other regions sources of sex differences are not known. Here we tested the hypothesis that both estradiol and sex chromosome complements modulate *Gh* mRNA and protein in a region-specific manner in the brain. We tested these two factors simultaneously and assayed three different brain regions: the hippocampus, the hypothalamus, and the cerebellum. We used the four core genotypes (FCG) mice, which have been previously utilized to compare sex chromosome versus gonadal sex as sources of sex differences. Adult FCG mice (XXF, XYF, XYM, XXM) were gonadectomized received a subcutaneous silastic implant containing 2 mg/ml 17β-estradiol benzoate in sesame oil (25 μl) or an empty implant. We found that estradiol increased *Gh* mRNA in all three brain regions examined. There was also a main effect of sex chromosomes in the hypothalamus, whereby mice with XY sex chromosomes had higher levels of GH mRNA and protein than mice with XX sex chromosome complements. However, protein extracted specifically from the arcuate nucleus of the hypothalamus did not recapitulate the gene expression pattern of the whole hypothalamus. In this area XX individuals had more GH protein than XY mice. Our data suggest nucleus-specific regulation of GH protein translation by sex chromosome complement in the hypothalamus.

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Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: 1R01AG031535-01A2

3R01AG031535-01A2S1

Title: 17β-estradiol regulates diurnal tail skin temperature of male rats

Authors: *I. J. MERCHENTHALER[1], M. V. LANE[2], S. VIECHWEG[3], J. A. MONG[3]
[1]Dept. of Epidemiology, Anatomy/Neurobiology, Univ. of Maryland, Sch. of Med., BALTIMORE, MD; [2]Epidemiology, Univ. of Maryland, Baltim, MD; [3]Pharmacol., Univ. of Maryland, Baltimore, MD

Abstract: Background: The primary therapy for alleviating menopausal symptoms in women, including hotflushes, depression/anxiety, impaired learning and memory, sleeplessness, etc., is estrogens. In hypogonadal men, testosterone but not the non-aromatizable dehydrotestosterone, also alleviates these symptoms indicating that even in men estradiol and not testosterone is responsible for these beneficial effects. One of the major hurtles in developing therapies for hot flushes is the lack of the most appropriate animal models. As only non-human primates flush similarly to menopausal women or andropausal men, they would be the best choice for use but due to ethical and economic reasons, primate model of hot flush arte not available. Although rodents do not flush, the ovarictomized, morphine-dependent rat is a frequently used model for drug testing. In this model, morphine withdrawal results in a 5-6 C elevation of tail skin temperature (TST) which is blocked by chronic estrogen treatment. This is a pharmacological model and therefore, attempts have been made to develop non-pharmacological ones. One of these is based on the diurnal changes is TST. This rhythmic change is present in intact but not in ovariactomized (OVX) rats. Estrogen given to OVX rats restores rhythmicity indicating the critical role of estrogen in TST regulation. Since estrogens reduce the elevation of TST in orchidectomized (ORDX), morphine-dependent rats, we thought to test if male rats also have a rhythmic, diurnal pattern of TST and if so, after does this diurnal pattern disappears and can subsequent estrogen treatment can restore the rhythmic pattern in male rats. Methods: Male rats were implanted with DSI telemetric transmitters and TST monitored for 6 days. Then, the rats were ORDXed and TST monitored for an additional 8 days At this point, Alzet pumps delivering physiological levels of 17β-estradiol were implanted subcutaneously and TST monitoring continued for additional 8 days. Results: Intact males, similarly to females, showed a diurnal, rhythmic pattern of TST which was lost in ORDX animals but restored with 17β-estradiol. Conclusion: These observations offer a new animal model of estrogen mediated TST regulation and as such, provides an additional toll to study the effects of estrogens not only in female but male animals. As our goal is to develop a brain-selective estrogen therapy for menopausal and andropausal hot flushes, this non-pharmacological model is of high value. Supported by: NIH grants 1R01AG031535-01A2 and 3R01AG031535-01A2S1

Disclosures: **I.J. Merchenthaler:** None. **M.V. Lane:** None. **S. Viechweg:** None. **J.A. Mong:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.05/NN14

Topic: E.01. Neuroendocrine Processes

Support: NSERC Grant

Title: Estradiol infusions into the dorsal striatum *in vivo* rapidly increase dorsal striatal dopamine levels

Authors: ***W. SHAMS**, C. SANIO, W. G. BRAKE
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Abstract: Systemic injections of 17β-estradiol (E2) in ovariectomized female rats rapidly enhance dorsal striatal dopamine (DA) release in response to amphetamine (AMPH; Becker & Rudick, 1999). Additionally, E2 rapidly (within 30 min) enhances AMPH-induced DA release (1990a; 1990b; Castner, Xiao, & Becker 1993). *In situ* studies show that this rapid effect of E2 occurs specifically within the dorsal striatum (Schultz et al., 2009). The present study investigated the *in vivo* effects of E2 infused into the dorsal striatum, substantia nigra or the medial prefrontal cortex on dorsal striatal DA levels. Rats were ovariectomized and implanted with a silastic tube containing 5% 17β-E2 in cholesterol, previously shown to mimic low physiological serum concentrations of 18-32 pg/ml. Single probe microdialysis was used to measure extracellular DA levels in the dorsal striatum. In addition, DA levels were measured subsequent to systemic injections of the indirect DA agonist, AMPH (0.5 mg/kg SC), administered simultaneously with E2 (0.544 µg/100µl) or its vehicle, cyclodextrin (0.520 µg/100µl). Local infusions of E2 into the dorsal striatum resulted in an AMPH-induced DA release in the dorsal striatum, comparison to its vehicle in accordance with previous *in situ* findings. Local infusions of E2 into the substantia nigra or the medial prefrontal cortex did not result in an enhancement of AMPH-induced DA levels in the dorsal striatum. These studies suggest that increases in dorsal striatal DA levels in response to systemic E2 are a consequence of E2 actions within the dorsal striatum itself.

Disclosures: **W. Shams:** None. **C. Sanio:** None. **W.G. Brake:** None.

Poster

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Rockefeller Research Grant RR024243

Title: Longitudinal characterization of neural estrogen signaling and neurotrophic changes in the Accelerated Ovarian Failure mouse model of menopause

Authors: *T. A. VAN KEMPEN[1], J. GORECKA[2], F. SOEDA[3], T. A. MILNER[1,2], E. M. WATERS[2]
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Abstract: Accelerated Ovarian Failure (AOF) can be induced in young mice (postnatal day 55) with low doses of 4-vinylcyclohexene diepoxide (VCD; 130 mg/kg), modeling the hormone changes observed across menopause. We assessed markers of synaptic plasticity in the hippocampus, anxiety-like behavior, and spatial learning longitudinally at four timepoints across the AOF model: premenopause (PRE; 24 days post-VCD administration), early perimenopause (EARLY; 52 days post-VCD administration), late perimenopause (LATE; 73 days post-VCD administration), and postmenopause (POST; 127 days post-VCD administration). As others have shown, VCD administration decreased ovarian follicle counts and increased acyclicity as the model progressed to POST, but with no impact on organ or body weights. The morphology of

Iba-1 immunoreactive microglia did not differ between VEH and VCD administered mice. Hippocampal PSD-95 levels were minimally altered across the AOF model, but decreased at POST in CA3b 24 hr after exogenous estradiol benzoate (EB; 0.25 mg/kg). In contrast, hippocampal phosphorylated AKT levels transiently decreased in PRE, but increased at POST after 24-hr EB in select sub-regions. Electron microscopy revealed fewer estrogen receptor α-containing dendritic spines and terminals in CA1 stratum radiatum at POST. mRNA levels of most BDNF exons (except V and VI) were lower in POST compared to ovariectomized mice. Exon V was sensitive to 24 hr EB administration in POST-VCD. Anxiety-like behavior was unaffected at any menopause phase. Spatial learning was unaffected in all groups, but POST-VCD mice performed below chance. Our results suggest that the AOF model is suitable for longitudinal studies of neurobiological changes across the menopause transition in mice. Our findings also point to complex interactions between estrogen receptors and pathways involved in synaptic plasticity.

Disclosures: **T.A. Van Kempen:** None. **J. Gorecka:** None. **F. Soeda:** None. **T.A. Milner:** None. **E.M. Waters:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.07/NN16

Topic: E.01. Neuroendocrine Processes

Title: Viaibility evaluation in treated swine semen with streptolysin o

Authors: ***B. DOMINGUEZ MANCERA**, M. VALDES CANO, M. BARRIENTOS MORALES, D. ROMERO SALAS, P. CERVANTES ACOSTA, A. HERNANDEZ BELTRAN Facultad de Veterinaria y Zootecnia, Univ. Veracruzana, Dept Fisiología, Veracruz, Veracruz., Mexico

Abstract: In recent years the semen cryopreservation protocols have been substantially improved with the use of permeable and nonpermeable diluents; however, these results are overshadowed by poor standing response to boar sperm freezing process. With the purpose of obtaining cell permeable membrane without alteration in function and viability has successfully experimented the use of streptolysin O (SLO) in various cells such as immune system, erythrocyte mouse sperm and oocytes. The aim of this study was to determine the effect on functional status and DNA integrity of porcine sperm cells treated with SLO for the opening of

transmembrane pores. The work was performed in the laboratories of Reproductive Biology, Cell Biology and Parasitology of the Diagnostic Unit "Mancisidor Augusto R. Ahuja" located in the Posta Zootécnica "Torreón del Molino." Nine ejaculates were obtained from 3 boars with 2 replicates for each, by gloved hand technique using a mannequin. Inclusion criteria were ejaculate mass motility values ≥ 3 and ≥ 70% motility individually. The chlortetracycline staining technique was used to determine the changes associated with sperm capacitation and acrosome reaction and acridine orange staining to assess the integrity of the nuclear chromatin. Incubation with SLO (Sigma-Aldrich) was performed at different concentrations (0.3, 0.6 and 1.2 IU / ml) for 5minutes to 37 ° C; pore sealing for fetal bovine serum (FBS) was used at 5%. After recovery of membrane integrity with a second evaluation Chlortetracycline acridine orange is performed. The data obtained were analyzed using the Kruscal Wallis H test program STATISTICA V7.01. It was found that the percentages of cells without training and without acrosome reaction remained above 75% in all treatments also no statistically significant difference ($p > 0.05$) was observed before and after pore sealing using SFB. By performing the analysis of the integrity of the nuclear chromatin significant differences ($p < 0.05$) were observed before and after sealing of pores in all treatments; however, the values of nuclear chromatin integrity were always above 95% so that it can be concluded that in this study using the SLO as a tool for membrane permeabilization not affect the functionality of the plasma membrane integrity or nuclear chromatin

Disclosures: **B. Dominguez Mancera:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACYT CB 169861. **M. Valdes Cano:** None. **M. Barrientos Morales:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACYT CB 169861. **D. Romero Salas:** None. **P. Cervantes Acosta:** None. **A. Hernandez Beltran:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Program#/Poster#: 448.08/NN17

Topic: E.01. Neuroendocrine Processes

Title: Viability of the following use of streptolysin O (SLO) as swine sperm cell permealizador agent

Authors: *M. BARRIENTOS, A. I. MONTALVO DIAZ, M. VALDES CANO, B. DOMINGUEZ MANCERA, C. LAMOTHE ZAVALETA, D. ROMERO SALAS
Univ. Veracruzana, Veracruz., Mexico

Abstract: Streptolysin O (SLO) permeabilized cells by interacting with the plasma membrane colesterol,and SLO monomers diffuse into theplane of themembrane to form dimers that act as points of crystallization to form complexes that grow in arcs and thenrings are continuous pores that permit access of molecules to the intracellular environment without damaging the membrane, pores having a diameter that could be larger than 30 nm so that theoretically the passage of large molecules through the membrane. In order to determine whether Streptolysin O adversely affects the viability, sperm capacitation and acrosome status of fresh porcine gametes or diluted and kept at 16 ° C for 24 hrs, this study was conducted. To which were obtained ejaculates of 5 boars (n = 15). Samples were obtained by the gloved hand technique and used ejaculated motile mass categorized as "good" and ≥ 70% progressive linear motion. Viability was assessed using the eosin nigrosin (EN) test, the capacitation sperm through thechlortetracycline (CTC) test and the acrosome reaction by the acridine orange(NA) test in the different treatments study: Fresh; Diluted; Fresh + 6% SLO, SLO Diluted + 6%, Diluted24 hrs and 24 hrs + 6% SLO. To evaluate the results STATISTICA v 7.0 for Windows StatSoft, Inc. (2004) was used, performing the No-parametric Wilcoxon test with a significance level of 0.05. The results show that treatment EN 24 hrs Diluted presented the lowest percentage of live sperm with 70% and + 6% Diluted SLO treatment the better viability to 79% ($p > 0.05$).The test results showed that CTC is the highest percentage of sperm with acrosome complete was Diluted to 6% + SLO treatment and showed the lowest percentage of Fresco treatment ($p<0.05$). Evaluation of chromatin integrity with NA in the different treatments found no statistical difference between treatments on the percentage of sperm with chromatin integrates the Diluted treatment having the lowest percentage at 93% and the largest 98% Fresh ($p > 0.05$). It can be concluded that using the SLO to 6%, no effect on sperm viability parameters

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Poster

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Topic: E.01. Neuroendocrine Processes

Support: NIH T32 AG020494

William & Ella Owens Medical Research Foundation

Texas Garvey Foundation

Title: The effect of androgens and oxidative stress on COX2 signaling in dopamine neurons

Authors: *S. HOLMES[1], R. NAZARLI[2], R. L. CUNNINGHAM[2]
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Abstract: Oxidative stress, inflammation, alpha synuclein, and the loss of dopamine neurons in the midbrain are hallmarks of Parkinson's disease (PD). Interestingly, men have a two fold higher risk for PD than women. While, the mechanisms underling this gender difference remain elusive, one possibility may be that androgens, the primary male sex hormone, is involved. Our previous studies have shown that androgens can increase oxidative stress and cell death in dopamine neurons. A possible mechanism underlying androgens negative effects on dopamine neurons may be the pro-inflammatory protein COX2, since COX2 can increase oxidative stress, alpha-synuclein accumulation, and dopamine neuronal loss. Therefore, we hypothesize that under oxidative stress conditions, the androgen, testosterone, will increase COX2 induced alpha-synuclein expression, leading to apoptosis in dopamine neurons. To test our hypothesis, we exposed a dopaminergic cell line (N27 cells) to a sublethal concentration of the pro-oxidant, tert-butyl hydrogen peroxide (H2O2) for 24 hrs and assessed the role of testosterone on COX2 signaling. Under low oxidative stress conditions, COX2 protein levels are low and alpha-synuclein expression and apoptosis are absent. However, under oxidative stress conditions, COX2, alpha synuclein, and apoptosis were increased, and these factors were exacerbated by testosterone. Our data shows that androgens may mediate the gender differences observed in PD by activating COX2 mediated inflammation and oxidative stress.

Disclosures: S. Holmes: None. **R.L. Cunningham:** None. **R. Nazarli:** None.

Poster

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Topic: E.01. Neuroendocrine Processes

Support: NIH R01 NS57823

P30 GM103398

Title: Neurokinin 3 receptor associates with histone acetyltransferase and nuclear proteins in paraventricular neurons of the hypothalamus

Authors: *A. THAKAR, F. W. FLYNN
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Abstract: Neurokinin 3 receptor (NK3R) is a G protein-coupled receptor that is widely expressed in brain and colocalizes with vasopressinergic neurons in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus. We demonstrated that in response to hyperosmolarity, membrane-bound NK3R are activated, internalized to the cytoplasm, and translocated to the nucleoplasm of PVN neurons. There, nuclear NK3R associated with acetylated histone 3 and histone 4. Acetylation of histones relaxes chromatin structure and allows the recruitment of transcription factors to gene promoters. Within the nucleus NK3R most likely interacts with other proteins and/or histone acetyltransferases (HATs) that enable interactions with chromatin because NK3R does not have HAT functional motifs or DNA binding motifs. Here we report that NK3R forms complexes with other nuclear proteins that possess HAT activity and with proteins involved in transcription regulation. Male Charles River Laboratory rats were maintained on water and chow (n=2), or 2% NaCl and chow for 3 days (n=2). The rats were sacrificed with lethal overdose of ketamine and the PVN was isolated. PVN tissue from rats in the same treatment group was combined, homogenized, and the nucleoplasm was separated from the sample containing the cytoplasm and plasma membrane. The nuclear sample was divided for either western blot or co-immunoprecipitation (Co-IP). The purity of the nuclear fraction was determined by probing the sample for secretory carrier membrane protein 5 (SCAMP5), a cytoplasmic protein. SCAMP5 was detected in cytoplasmic/membrane fraction, but not in nucleoplasm. NK3R was detected in the cytoplasmic/membrane and nuclear fractions of both control and salt loaded rats, but there was more detectable NK3R in the nuclear sample from the salt loaded rats. A selective NK3R antibody (raised in sheep) was conjugated to magnetic beads and after elution the samples were run on SDS PAGE, transferred, and probed for NK3R (antibody raised in sheep), and then the following nuclear proteins (all antibodies raised in rabbit): P300/CBP-associated factor (PCAF), β-actin, lamin B2, and acetyl-H3K9. In both groups, co-IP results showed that NK3R pulled down PCAF, β-actin, lamin B2, and acetyl-H3K9. As a negative control, membranes were probed for SCAMP5 and it was not present in the pulled down samples. These data suggest that NK3R forms complex with PCAF to acetylate histones, and β-actin and lamin B2, which are directly involved in transcription initiation/regulation. Hence, within the nucleus NK3R forms complexes with multiple proteins that collectively modify chromatin structure and affect transcription.

Disclosures: A. Thakar: None. **F.W. Flynn:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant MH090224

NSF Grant IOS-1022148

Title: Pubertal differences in stress-induced oxytocin and vasopressin responses in male rats

Authors: S. MINHAS, J. FLORES-GALDAMAZ, *R. D. ROMEO
Psychology and Neurosci. and Behavior Program, Barnard Col. of Columbia Univ., NEW YORK, NY

Abstract: Though adolescence is a time in development associated with many changes in neurobehavioral function, it is also a significant period of developmental vulnerability, marked by physiological and psychological disorders, such as anxiety, depression, drug abuse, and obesity. Recent human and animal studies indicate that pubertal exposure to stress is a particularly relevant environmental factor that contributes to these morbidities, yet the mechanisms through which stress mediates the increase in dysfunction is not understood. Notably, puberty is marked by significant changes in hormonal stress reactivity, which may contribute to these vulnerabilities. Studies have shown that periadolescent animals display greater stress-induced hypothalamic-pituitary-adrenal (HPA) axis responses than adults. Specifically, adrenocorticotropin (ACTH) and corticosterone (CORT) responses remain elevated for twice as long in prepubertal compared to adult rats. In addition to the HPA axis, the hypothalamo-neurohypophyseal tract (HNT) is also activated in response to stress. In adults, stress-induced activation of this system results in secretion of oxytocin (OXY) and vasopressin (AVP) from neurons in the magnocellular subdivision in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) through the posterior pituitary into the bloodstream. The response of the HNT of prepubertal animals is presently unknown. Given the influence of these hormones on a variety of physiological parameters and emotional and social behaviors, the following study investigated the stress-induced OXY and AVP hormonal and neural responses in prepubertal (30 days of age) and adult (70 days of age) male rats exposed to a 30 min session of restraint stress. Though we found the well-established protracted ACTH and CORT response in prepubertal animals, only adults showed significant stress-induced OXY and AVP hormonal responses. We

also found that no clear relationship exists between the number of OXY and AVP neurons in the PVN and SON and these age-dependent changes in the stress responsiveness of the HNT. These data indicate that the pattern of pubertal-related changes in hormonal stress responsiveness is dependent on the neuroendocrine system examined. Moreover, given the significant increase in stress-related vulnerabilities during adolescence, it will be imperative to further explore these changes in stress reactivity and what role they may play in adolescent mental and physical health.

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Poster

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Topic: E.01. Neuroendocrine Processes

Support: Instituto Nacional de Psiquiatría RFM-NC093290.0

Title: Effect of polibrominated diphenyl ethers (PBDEs) on nitric oxide synthase, oxytocin and vasopressin of the hypothalamic supraoptic and paraventricular nuclei of lactating rats

Authors: *E. SANCHEZ-ISLAS, M. ÁLVAREZ-GONZÁLEZ, S. MUCIO-RAMÍREZ, M. LEÓN-OLEA
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Abstract: The magnocellular and parvocellular neurons adopt different expression patterns of nitric oxide synthase (NOS), oxytocin (OXT) and vasopressin (AVP) in response to relevant stimuli and physiological processes. During lactation there is an increase of these three substances in the hypothalamic nuclei where they are produced. These substances are damaged by environmental organobromine pollutants like PBDEs, used as flame retardants and classified as endocrine disruptors. Previously we reported alterations in the NADPH-d activity and NOS and AVP immunoreactivity (IR) in the paraventricular (PVN) and supraoptic nuclei (SON) of pup rat brains perinatally exposed to DE-71. The aim of this study was to determine the effects of PBDE -71 and -79 on the activity of NOS and the nNOS, OXT and AVP-IR in the PVN and SON of mother rats in the final period of lactation (lactation day 22, LD 22). Adult pregnant Wistar rats were used, they were given orally DE- 71 or DE-79 at doses of 1.7, 10.2 and 30 mg / kg / day dissolved in corn oil (vehicle) in popcorn from gestational day 6 to postnatal day 21.

Control pregnant rats received only vehicle. On the LD 22, two groups of mother rat were formed. One group was perfused with 4% paraformaldehyde previous anesthesia with sodium pentobarbital (30 mg/kg). Brains were removed, postfixed in the same fixative (2-4 h, 4°C) and cryoprotected with 30% sucrose. 30µm coronal sections were cut on a cryostat (-18°C). The sections were processed for the NADPH-d histochemistry and nNOS, OXT and AVP immunohistochemistry. Photomicrographs were taken from the PVN and SON and evaluated the integrated optical density of the NADPH-d histochemestry and the nNOS, OXT and AVP-IR. The second mothers group, after anesthesia, was decapitated. The brains were quickly frozen and the PVN and SON were used for Western blot assay. The data were statistically analyzed with a one way ANOVA. The immunohistochemistry results showed that treated mothers with DE-71 significantly increased the nNOS-IR in both PVN and SON nuclei; it was more remarkable in the 30 mg dose. While with 30 mg of DE-79 showed a significant decrease. The OXT-IR present a significant decrease in PVN and SON with both toxics, it was more evident with DE-79 at 30 mg dose. The VP-IR in the mother rats treated with DE-79 at a 30 mg dose, showed a significant increase in both cerebral nuclei. The Western blot analysis showed similar results that immunohistochemical study. These results suggest that exposure to toxic PBDEs can cause alterations in the production of NO, OXT and AVP in the hypothalamic nuclei, which may have an impact on functions of these cerebral nuclei, such as lactation and osmoregulation.

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Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Title: Expression of mineralocorticoid receptor and 11ß-hydroxysteroid dehydrogenase type 2 in the hypothalamic supraoptic nucleus of various rat strains

Authors: *M. HAQUE, A. SABRIN, R. A. WILSON, N. E. J. WANDREY, N. MILLS, R. TERUYAMA
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Abstract: Hypertension is a major health concern, but its etiology is not well understood. An accumulating body of evidence suggests that activity of the mineralocorticoid, aldosterone, in the

brain via mineralocorticoid receptor (MR) plays an important role in the development of hypertension. MR was recently found in vasopressin (VP) and oxytocin (OT) synthesizing magnocellular neurosecretory cells (MNCs) in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) in the hypothalamus. Abnormally elevated plasma levels of VP and OT were observed in hypertensive human and animal models of hypertension, and excessive VP is implicated in modulating a number of processes occurring in the pathogenesis of cardiovascular diseases. These findings suggest an involvement of aldosterone in the mechanism controlling the release of these hormones. Aldosterone exerts its biological effect via MR; however, glucocorticoids also have high binding affinity to MR at substantially higher concentrations than does aldosterone. The enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), converts glucocorticoid into inactive metabolites that increases aldosterone selectivity. The present study was conducted to elucidate whether 11β-HSD2 is expressed in MR expressing VP and OT MNCs. Double immunofluorescence confocal microscopy demonstrated that MR and 11β-HSD2 immunoreactivities were found in both VP- and OT-immunoreactive MNCs. *In situ* hybridization also visualized 11β-HSD2 mRNA in MNCs. Lastly, single-cell RT-PCR detected MR and 11β-HSD2 mRNAs from cDNA libraries derived from single identified VP and OT MNCs. Co-localization of MR and 11β-HSD2 in VP and OT MNCs suggests that aldosterone directly affects the activity of these cells through MR. In addition, MR immunostaining intensity and gene expression in the SON were compared among various popular rat strains [Wister-Kyoto (WKY), Spraque-Dawley (SD), Wister, Dahl salt-sensitive (DSS), and spontaneously hypertensive (SHR)] for cardiovascular research. The MR immunostaining intensity of the SON from WKY and SHR was significantly greater than that of other strains. Semi-quantitative RT-PCR also detected significantly greater MR mRNA expression in the SON of WKY compared to that of Wister, SD, and DSS. These results provide critical information to researchers who utilize these strains of rats in cardiovascular research.
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Disclosures: **M. Haque:** None. **A. Sabrin:** None. **R.A. Wilson:** None. **N.E.J. Wandrey:** None. **N. Mills:** None. **R. Teruyama:** None.

Poster

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HD23315-22

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Pew Scholarship

Title: Patterns of oxytocin receptor expression in the rodent central nervous system

Authors: *M. MITRE, B. J. MARLIN, S. NORDEN, R. C. FROEMKE, M. V. CHAO
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Abstract: Oxytocin is an essential neuropeptide that regulates social behaviors such as maternal care and parent-infant bonding. It is synthesized in the paraventricular nucleus and supraoptic nucleus of the hypothalamus and is released both peripherally and centrally. Peripheral release into the systemic circulation is responsible for its classical physiological functions of milk ejection and initiation of parturition, whereas central release seems to modulate social cognition in animals, and has been implicated in maternal behavior, aggression, anxiety, fear, interpersonal trust, autism spectrum disorders, schizophrenia, and depression. It is believed that the forebrain distribution of oxytocin receptors is responsible for its effect in modulating certain social behaviors and cognition; however, the precise expression pattern of oxytocin receptors and the downstream effects of oxytocin receptor signaling both remain unclear. Here we examine the distribution of oxytocin receptors in the rodent brain and their mechanism of action. To follow the distribution of the oxytocin receptor, we generated antibodies against unique sequences of the receptor. Using immunohistochemistry, we identified oxytocin receptors in the lateral septum, cortex, medial amygdala, hippocampus, and hypothalamus of male and female mice. No labeling was detected in oxytocin receptor knockout animals. We also co-stained for oxytocin peptide and used tract-tracing methods (e.g., pseudorabies virus) to determine the projection pattern of oxytocin fibers in relation to oxytocin receptor distribution. Work in the Froemke lab has determined that parental experience interacts with oxytocin-based neuromodulation to affect the response of neural circuits of the adult mouse auditory cortex to neonatal vocalizations (Marlin et al. SFN abstracts 2014). In particular, the left auditory cortex is functionally sensitive to oxytocin for the expression of pup retrieval behaviors in females. Interestingly, we have found that oxytocin receptor expression in left auditory cortex is higher than right, and this might be part of the cellular mechanism for specialized processing of pup calls. Co-staining with inhibitory neuron markers revealed that many oxytocin receptor expressing neurons were somatostatin-positive or parvalbumin-positive interneurons. This suggests that oxytocin modulation in cortex (and perhaps other brain regions) is important for control of local inhibition, providing a potential mechanism by which oxytocin regulates perceptual salience of social information.

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Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: DK043200

Title: Dynorphin modulation of physiological prolactin surges

Authors: *A. M. STATHOPOULOS[1], F. S. NENNINGER[2], J. ARIAS-CRISTANCHO[2], R. CRISTANCHO-GORDO[2], C. V. HELENA[2], A. E. GONZALEZ-IGLESIAS[2], R. BERTRAM[2]
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Abstract: Prolactin (PRL) is an anterior pituitary hormone important for fertility and reproductive success. PRL is primarily regulated by inhibition from hypothalamic dopamine (DA) and DA levels must typically drop in order for large amounts of PRL to be released. Endogenous opioids are believed to be necessary for the inhibition of DA that allows the surge in PRL secretion during pregnancy, delivery, and lactation. During early rodent pregnancy or pseudopregnancy induced by cervical stimulation (CS), female rats exhibit twice-daily surges in PRL release, one nocturnal and one diurnal surge. We show that i.c.v norbinaltorphimine (nor-BNI), a kappa opioid receptor antagonist, blunts the nocturnal PRL surge in cervically-stimulated ovariectomized rats (OVX-CS), similar to a previous report in intact, pregnant animals. This result suggests that endogenous dynorphin, perhaps coming from the KNDy neurons of the arcuate nucleus, is a factor in the control of PRL release during pregnancy and pseudopregnancy. On the afternoon of proestrus in the rat, PRL levels, along with many other hormones, rise to help prepare the female for mating and potential pregnancy. This single diurnal rise in PRL can be reproduced in OVX rats treated with replacement estradiol (OVE). In OVE rats, nor-BNI administration into the lateral ventricle immediately before the expected PRL surge did not change DA and PRL profiles, suggesting that the diurnal estradiol-induced surge is generated by a different mechanism than the nocturnal CS-induced PRL surge. Future tests will explore the effect of kappa opioid blockade on the diurnal PRL surge in OVX-CS animals. These results will provide crucial information on the mechanisms employed in the generation PRL surges induced by cervical stimulation and estradiol.

Disclosures: A.M. Stathopoulos: None. **F.S. Nenninger:** None. **J. Arias-Cristancho:** None. **R. Cristancho-Gordo:** None. **C.V. Helena:** None. **A.E. Gonzalez-Iglesias:** None. **R. Bertram:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.16/NN25

Topic: E.01. Neuroendocrine Processes

Support: JSPS KAKENHI 23580404

Title: Induction of c-Fos expression following heat exposure in the hypothalamus of neonatal chicks

Authors: *S.-I. KAWAKAMI, S. SUMIHARA, Y. KUROSAWA, T. BUNGO
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Abstract: Exposure to high environmental temperature is known to negatively affect animal growth. One of the factors has been that heat stress strongly suppresses feeding behavior of animals, the brain mechanisms regulating heat stress-induced feeding suppression remain unknown. We have previously shown that central administration of corticotropin-releasing hormone (CRH) antagonist diminishes heat stress-induced suppression of feed intake in neonatal chicks. The result suggests that CRH neurons localized mainly in the hypothalamic paraventricular (PV) nucleus (n.) are activated by heat stress to induce stress responses such as feeding suppression, but no information is currently available about which brain areas are activated by heat stress in the avian central nervous system. Therefore, the aim of the present study was to examine the immunohistochemical localization of c-Fos immunoreactivity in the hypothalamus of heat stressed neonatal chicks. A total of 10 male layer chicks (6- or 7-day old) were assigned to one of 2 thermal condition groups, 30°C as control, and 37°C as constant heat stress. After 6 h of the treatments, the chicks were anesthetized with sodium pentobarbital and then perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde. The brains containing hypothalamus were coronally cut using a cryostat to make 20 μm-frozen sections. The sections were incubated with mouse primary antibody against c-Fos (Santa Cruz), then with Alexa Fluor 568 goat anti-mouse IgG (H+L) antibody (Life Technologies). The immunostained sections were mounted with VECTASHIELD Mounting Medium (Vector). Digital images were captured using fluorescence microscope (Nikon) and analyzed with image processing software (ImageJ). Heat stress significantly elicited c-Fos expression in the preoptic region of the hypothalamus such as median preoptic n., anteromedial preoptic n., medial preoptic n. and n. of the hippocampal commissure. c-Fos expression was also increased in the

parvicellular part of the PV n. These data suggest that heat stress activates various preoptic n. to receive and process thermal information from peripheral, and PV n. to induce a variety of stress responses such as feeding suppression in the hypothalamus of neonatal chicks.

Disclosures: **S. Kawakami:** None. **S. Sumihara:** None. **Y. Kurosawa:** None. **T. Bungo:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant P01 HD075750

Title: fMRI in the prairie vole: methods development for translational social neuroscience

Authors: ***W. KENKEL**, J. R. YEE, K. MOORE, P. KULKARNI, S. CARTER, C. F. FERRIS
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Abstract: Non-invasive imaging of brain activity offers a powerful approach to better understand socioaffective processes for which the prairie vole (Microtus ochrogaster) has been long studied. Here, we report on the development of functional neuroimaging methods in awake, un-anesthetized prairie voles. Voles were imaged inside a 7T magnet, which acquires functional images of the entire brain every 6 seconds by using a RARE pulse sequence (FOV 2 cm, 96 x 96 matrix). Beginning with a fully segmented brain atlas with 116 discrete regions specifically developed for the vole, we used blood oxygen level dependent (BOLD) changes to index neural reactivity to a variety of provocations. In the first proof-of-principle experiment, voles exhibited the expected global increase in BOLD activity following hypercapnic challenge (5% CO2), demonstrating the validity of the imaging approach for the assessment of cerebrovascular reactivity. Data on BOLD reactivity to various other provocation paradigms (e.g. social stimuli and oxytocin administration) will also be presented. Unexpectedly, we have found voles to differ from rats in terms of their acclimation to the conditions of restraint necessary for stable imaging. Whereas rats gradually habituate to restraint, voles do not and even show heightened heart rate and diminished parasympathetic drive at baseline following 5 days of daily 15 minute restraint sessions. Furthermore, voles display greater motion following repeated restraint, which has led us to conclude that repeatedly restraining the vole may be counter-productive for reliable imaging. This could be due to species differences in reactivity, possibly related to the

domestication of the traditional laboratory rat, and/or the specifics of the restraint hardware specially developed for vole imaging. Overall, vole imaging holds great promise for the future of translational approaches to social neuroscience. We expect to expand these methods to include diffusion tensor imaging (DTI) and functional connectivity, in addition to investigations of social and neuroendocrine processes.

Disclosures: W. Kenkel: None. **C.F. Ferris:** None. **J.R. Yee:** None. **P. Kulkarni:** None. **S. Carter:** None. **K. Moore:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.18/NN27

Topic: E.01. Neuroendocrine Processes

Title: The effects of running wheel access on the symptoms of type-2 diabetes in TallyHo/JngJ mice

Authors: *N. NASCIMENTO, D. AMARAL, K. CARLSON, G. NASH, D. PYNE, J. A. SEGGIO
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Abstract: Type 2 Diabetes mellitus is the most common form of human diabetes, accounting for about 90% of cases and affecting about 23 million Americans. It is often accompanied by hyperglycemia and obesity. Diabetes has been linked to circadian rhythms (a 24-hour long daily biological clock) as it can affect critical clock genes and disruptions in the rhythm can lead to increased possibility of developing the disorder. The pancreas has also been shown to release insulin on a daily rhythm and alterations to the biological clock leads to altered insulin production and release. This study looked at the effects of voluntary exercise in the form of access to a running-wheel on common symptoms of diabetes, including fasting blood glucose levels and increased body weight. Five-week old C57BL6/J (B6) and TallyHo/JngJ (TH) mice were kept in 12:12 LD cycle, and given standard chow and water ad libitum. Half of each strain received access to a running wheel while the other half were placed into a cage which can monitor home cage locomotor activity, but without a running wheel. Weekly measurements of body weight and water consumption were recorded. In addition, a 12-hour fasting glucose tolerance test with 30, 60, and 120 min time-points was conducted every four weeks starting at age-week eight. Surprisingly, neither TH nor B6 mice exhibited reduced body mass or 12-hour

fasting glucose levels when given access to running wheel compared to mice without running wheels. However, access to a running-wheel may reduce diabetes symptoms in young TH mice but not in older mice, as eight-week old TH mice with access to a running-wheel exhibited lower glucose levels during the glucose tolerance test compared to eight-week old TH mice without running-wheel access. In contrast, twelve-week old TH mice showed no difference in glucose tolerance when given access to a running-wheel, indicating that older mice are less sensitive to the effects of exercise to a running-wheel. Future studies will investigate the effects of chronobiological disruption, such as simulated jet-lag, on glucose-tolerance and obesity in both type-2 and non-type-2 diabetic mouse models.

Disclosures: **N. Nascimento:** None. **D. Amaral:** None. **K. Carlson:** None. **G. Nash:** None. **D. Pyne:** None. **J.A. Seggio:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.19/NN28

Topic: E.01. Neuroendocrine Processes

Title: Galanin-like peptide (GALP) have the anti-obesity effect and control of energy metabolism via the sympathetic nervous system

Authors: S. HIRAKO[1], *H. KAGEYAMA[2], F. TAKENOYA[3], N. WADA[1], A. KIMURA[1], M. OKABE[4], S. SHIODA[1]
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Abstract: Introduction: GALP is produced in neurons in the hypothalamic arcuate nucleus and is well known as a neuropeptide regulating feeding behavior and energy metabolism. In this study we will show its function on lipid metabolism in the liver. Methods: Mice were i.c.v. injected saline or GALP (2nmol), and removal of the liver and adipose tissue at 100 minutes after the administration of GALP. Then, we studied hepatic mRNA expression by use of real-time quantitatively PCR analysis. Next, we examined the effect of GALP on lipid metabolism when its effect was blocked by the sympathetic nervous system by guanethidine treatment. Moreover, to investigate the anti-obesity effect of chronic administration of GALP, mice were fed a high fat diet to induce obesity and were intranasal administrated of GALP for 1 week. Results: The respiratory exchange ratio (RER) of GALP group was lower than the saline group at 1 hour after

administration. In the GALP treated group, fatty acid synthesis-related gene mRNA levels were decreased and fatty acid oxidation-related gene mRNA levels were increased in liver. RER was reduced by GALP administration, but these changes were not observed in the guanethidine treatment. In adipose tissue, the mRNA levels of HSL ATGL which involved in lipolysis, were increased in the GALP group compared with the saline group. By GALP administration of 1 week, body weight gain was decreased compared with the saline group. In addition, WAT weight was decreased by GALP treatment. Conclusion: In this study demonstrates that anti-obesity effect of GALP for central and intranasal administration, and its action was through the sympathetic nervous system.

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Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant RO1DK080441

Title: Neuropeptide Y regulates adrenal function during the counter-regulatory response to recurrent hypoglycemia

Authors: Y. MA, Q. WANG, *M. D. WHIM
Cell Biol. and Anat., LSUHSC, New Orleans, LA

Abstract: The counter regulatory response (CRR) is a hormonal and neuroendocrine mechanism that is essential in restoring blood glucose levels following the onset of hypoglycemia. By suppressing insulin and potentiating glucagon release, the CRR contributes to recovery of euglycemia. In type I diabetes, effective control of insulin and glucagon release is impaired and the restoration of euglycemia is critically dependent on epinephrine release from the adrenal gland. However following recurrent episodes of hypoglycemia (which are common in the diabetic state), the ability to evoke epinephrine release becomes progressively worse leading to an elevated risk of severe hypoglycemia. The reasons for the failure of the CRR following recurrent hypoglycemia are not clear but could involve central or peripheral mechanisms (for example an adrenal impairment of epinephrine synthesis or release). To examine this issue we

quantified the adrenal expression of tyrosine hydroxylase (TH; the rate limiting enzyme for catecholamine synthesis) in (i) euglycemic mice; (ii) mice exposed once to insulin-induced hypoglycemia (IIH) and (iii) mice subjected to three daily episodes of IIH. We first confirmed that IIH produced circulating glucose levels of less than 60 mg / dL and that the hypoglycemia-induced rise in urine epinephrine levels was blunted after recurrent IIH. Quantification of adrenal TH-ir showed that although one episode of IIH resulted in an increase in TH expression, this effect was blunted after three episodes of hypoglycemia. In contrast, the adrenal levels of neuropeptide Y were increased after single and repeated episodes of IIH. We have previously shown that neuropeptide Y is an adrenal co-transmitter that inhibits TH expression via Y1 receptors (Wang et al, 2013). When the levels of TH were quantified in neuropeptide Y knockout mice we found they were now elevated after both single and recurrent episodes of IIH. To determine whether this effect was mediated by Y1 receptors, BIBP3226, a Y1 antagonist that does not cross the blood brain barrier was injected (1 mg / kg i.p.) prior to IIH in wild type mice. In these animals, the levels of TH-ir were elevated after single and repeated episodes of IIH, mimicking the results seen in the neuropeptide Y knockout mice. When we quantified gluconeogenesis in wild type mice using the pyruvate tolerance test there was no difference between the three experimental groups. Thus the CRR downstream of the adrenal appears intact. These results suggest that impairment of the CRR involves a peripheral defect in adrenal signaling that is due to an activity- and neuropeptide Y-dependent inhibition of TH synthesis and adrenal secretory capacity.

Disclosures: **Y. Ma:** None. **Q. Wang:** None. **M.D. Whim:** None.

Poster

448. Neuroendocrine Anatomy and Physiology

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Title: Glucose-dependent compensation of deficient insulin secretion in mutant mice lacking the cdk5 activator p39

Authors: *C. I. BARK[1], M. SKELIN[2], C. MATTSSON[3], S. A. MANDIC[3], Å. MATTSSON[3], T. DARAIO[3], M. JEVSEK[2], I. VALLADOLID ACEBES[3], T. HÖKFELT[3], K. BRISMAR[3], M. S. RUPNIK[2], P.-O. BERGGREN[3]
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Abstract: Cyclin-Dependent Kinase 5, Cdk5, requires association with either the p35 or p39 proteins in order to become an active kinase. Cdk5 is in particular active in post-mitotic cells and regulates numerous functions in the nervous system; neuronal migration, axonal guidance and synaptic plasticity. However, Cdk5 has also been implicated in roles outside of the nervous system, e.g. in vesicular transport, apoptosis and cell adhesion. In addition, Cdk5/p39 has been demonstrated to stimulate secretion of insulin from pancreatic β-cells *in vitro,* at least partly by promoting the phosphorylation of Munc18-1 in a step connected to insulin granule fusion with the plasma membrane. **Aim:** The current objective was to investigate the effect of p39 on insulin secretion and glucose homeostasis *in vivo*. **Methods:** We have performed *in vivo* glucose homeostasis and insulin secretion analyses on genetically engineered mice where the gene for the Cdk5 activator p39 was disrupted. In addition, isolated islets and pancreatic slices from p39-deficient and control littermates have been subjected to electro-physiological recordings. **Results:** The electro-physiological recordings from p39-deficient pancreatic slices demonstrated an impaired initial run up of Ca^{2+}-dependent exocytosis. Surprisingly, there were no significant impairments either of blood glucose handling or release of insulin during glucose tolerance tests on live mice. However, blockage of the K_{ATP} channel (with glibenclamide), uncoupling glucose metabolism from Ca^{2+}-dependent exocytosis, resulted in reduced insulin secretion in p39-deficient mice compared to wild-type littermates. Further, p39-deficient pancreatic slices subjected to glucose stimulation demonstrated an earlier onset of electrical activity in β-cells compared to control. **Conclusions:** The Cdk5 activator p39 promotes insulin secretion, however, in the absence of p39 *in vivo* this impairment is compensated for by a glucose-dependent mechanism. The rescue of the deficient insulin secretion by glucose could act either through increased ATP-sensitivity of K_{ATP} channels, or increased activity of GLUT2 or glucokinase.

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Poster

448. Neuroendocrine Anatomy and Physiology

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 448.22/NN31

Topic: E.01. Neuroendocrine Processes

Support: Pekary Trust

Title: Ketamine modulates TRH and TRH-like peptide turnover in brain and peripheral tissues of male rats

Authors: *A. E. PEKARY[1], A. SATTIN[2], R. L. LLOYD[3]
[1]Res., [2]Psychiatry, VA Greater Los Angeles Hlthcare Syst, Los Angeles, CA; [3]Psychology, Univ. of Minnesota, Duluth, MN

Abstract: Major depression is the largest single healthcare burden with treatments of slow onset and often limited efficacy. Ketamine, a NMDA antagonist used extensively as a pediatric and veterinary anesthetic, has recently been shown to be a rapid acting antidepressant, making it a potential lifesaver for suicidal patients. Side effects and risk of abuse limit the chronic use of ketamine. More complete understanding of the neurobiochemical mechanisms of ketamine should lead to safer alternatives. Some of the physiological and pharamacological actions of ketamine are consistent with increased synthesis and release of TRH (pGlu-His-Pro-NH_2), and TRH-like peptides (pGlu-X-Pro-NH_2) where "X" can be any amino acid residue. Moreover, TRH-like peptides are themselves potential therapeutic agents for the treatment of major depression, bipolar disorder, epilepsy, Alzheimer's and Parkinson's diseases. For these reasons, male Sprague-Dawley rats were anesthetized with 162 mg/kg ip ketamine and then infused intranasally with 20 µl of sterile saline containing either 0 or 5 mg/ml Glu-TRH. One, 2 or 4 h later, the brain levels of TRH and TRH-like peptides were measured in various brain regions and peripheral tissues. At 1 h in brain following ketamine only, the levels of TRH and TRH-like peptides were significantly increased in 52 instances (due to increased biosynthesis and/or decreased release) or decreased in 5 instances. These changes, listed by brain region in order of decreasing number of significant increases (↑) and/or decreases (↓), were: hypothalamus (9↑); piriform cortex (8↑); entorhinal cortex (7↑); nucleus accumbens (7↑); posterior cingulate (5↑); striatum (4↑); frontal cortex (2↑,3↓); amygdala (3↑); medulla oblongata (1↑,2↓); cerebellum (2↑); hippocampus (2↑); frontal cortex (2). The corresponding changes in peripheral tissues were: adrenals (8↑); epididymis (4↑); testis (1↑,3↓); pancreas (1↑); prostate (1↑). We conclude that TRH and TRH-like peptides may be downstream mediators of the rapid antidepressant actions of ketamine.

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Poster

448. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant 5T32DK091317-03

Title: Environmental impacts on imprinted monoamine genes

Authors: ***P. J. BONTHUIS**[1], E. FERRIS[1], C. T. GREGG[1,2]
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Abstract: Imprinted gene expression is an epigenetic phenomenon where the level of allelic gene expression depends on whether an allele is inherited from the mother or the father. Our lab sequenced the transcriptome (RNAseq) of F1 hybrid mice, produced by reciprocal crosses of Castaneous and C57Bl/6 inbred lines, to discover hundreds of novel imprinting effects in the brain by measuring maternally and paternally derived transcripts by single nucleotide polymorphisms (SNPs). RNAseq analysis of several central and peripheral tissues found the arcuate nucleus (ARN) of the hypothalamus to be particularly enriched in the number of genes that have parent-of-origin effects on gene expression. Neurons of the ARN regulate feeding and energy homeostasis, and exhibit neuroendocrine control of hormone secretion from the pituitary. Using gene network analysis, we discovered a gene pathway of novel imprinted genes involved in monoamine neurotransmitter signaling in the arcuate: namely, genes involved in dopamine and serotonin biosynthesis and signaling. Dopamine and serotonin both regulate energy homeostasis, feeding, and neuroendocrine responses. In addition, tuberoinfundibular dopamine (TIDA) neurons in the dorsal medial arcuate release dopamine into the median eminence to inhibit lactotrophs in the anterior pituitary from secreting prolactin into the blood. Prolactin is involved in stimulating mammary gland development and lactation, and promoting food intake in rodents. To explore potential functional roles of imprinting effects on monoamine signaling in the ARN, I used F1 female hybrid mice in the following two experiments: 1) altered energy homeostasis by fasting mice and measuring effects on monoamine gene expression and imprinting; and 2) measuring monoamine gene expression and imprinting levels between lactating and virgin female mice. Adult female F1 hybrid mice fasted for 24-hours showed gene

expression changes in the ARN consistent with reduced dopamine signaling compared to ad libitum fed controls; but, the maternal to paternal allelic expression bias remained relatively stable. In general, lactating females also had reduced expression of dopamine synthesis genes compared to virgin females. However, the maternal to paternal allelic expression biases were affected in a gene specific manner in lactating females, with some genes stable and others increased in the parental expression bias. These results demonstrate that imprinting effects in the brain can be both stable and dynamic depending on the context of environmental experiences.

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Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

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Fonds Spéciaux Ulg 12/06

CdB is Non FRIA Ulg grantee

CAC is FRS-FNRS Research Associate

Title: Glutamate controls brain estrogen synthesis during sexual interactions

Authors: C. DE BOURNONVILLE[1], N. AOURZ[2], A. VAN EECKHAUT[2], I. SMOLDERS[2], G. F. BALL[3], *J. H. BALTHAZART[1], C. A. CORNIL[1]
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Abstract: Besides their long-lasting effects mediated by a modulation of gene transcription, brain-derived estrogens can rapidly regulate (within minutes) reproductive behaviors. *In vitro*, the activity of aromatase (AA), the enzyme responsible for the conversion of androgens into estrogens, is also regulated on a similar short time-scale, via phosphorylation of the enzyme resulting from changes in neuronal activity or glutamate release. Acute changes in AA have been documented *ex vivo* in specific brain regions following exposure to social or stressful stimuli but

the mechanism underlying these regulations is not known. To investigate whether glutamate is implicated in these rapid changes in AA, male quail received a unilateral injection of kainate in the medial preoptic nucleus (POM). The left and right preoptic areas were collected 20 min later and assayed separately by the tritiated water technique for AA. As shown previously in preoptic explants maintained *in vitro*, AA was down-regulated in the kainate-injected hemisphere as compared to the non-injected side. To determine whether the decline in AA detected in the POM after a sexual interaction could be mediated by an increased release of glutamate in this region, extracellular glutamate concentration was measured by *in vivo* microdialysis with a probe implanted in the POM of sexually mature males. Dialysate was collected every 3 minutes over three periods of 15 min when the male was (1) alone, (2) allowed to freely copulate with a female and (3) alone again. A transient rise in extracellular glutamate concentration was observed specifically and immediately after the expression of cloacal contact movements, when semen is transferred to the female. Glutamate returned to a basal level after the female was removed. Together, these results indicate that the mechanism of acute regulation of aromatase activity by glutamate identified *in vitro* is potentially responsible for the acute regulation of the enzyme observed *in vivo* following copulation. As rapid changes in brain estrogen synthesis and its actions are apparently related to the control of sexual motivation rather than sexual performance, follow up experiments should now determine whether the release of glutamate in the POM occurs in parallel with an increase in motivation or follows the termination of the copulatory sequence.

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Poster

449. Male Sexual Behavior

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Support: NIH Grant MH50388

Fonds Spéciaux Ulg 12/06

CAC is FRS-FNRS Research Associate

Title: Preoptic aromatase neurons are activated by copulation in male quail

Authors: M.-A. CEULEERS[1], V. J. CHRISTOPHE[1], G. F. BALL[2], J. BALTHAZART[1], *C. A. CORNIL[1]
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Abstract: The full activation of male sexual behavior by testosterone requires its aromatization into estrogens in the brain. Brain aromatase activity is controlled by two types of mechanisms: (1) in the long term, the concentration of the enzyme is increased by the transcriptional activity of androgens and estrogens, and (2) in a shorter time frame, the enzyme kinetics is acutely modulated by neuronal activity through calcium-dependent phosphorylations. It has recently been shown that both observing a female or copulating with her rapidly inhibits aromatase activity in specific brain regions including the medial preoptic nucleus (POM). Moreover, quantitative studies of the expression of the immediate early gene (IEG) c-Fos indicated that appetitive and consummatory sexual behaviors activate distinct subregions of the POM, including areas characterized by a dense expression of aromatase. Previous studies suggested however that very few aromatase-immunoreactive (ARO-ir) neurons show an enhanced c-Fos expression following copulation in the POM, while conflicting results were reported for the medial bed nucleus of the stria terminalis (BST). The present study was therefore designed to determine whether changes in aromatase activity induced in the POM and BST by visual or sexual interactions with a female are associated with an activation of ARO-ir neurons as measured by enhanced expression of the IEG Zenk (also known as Zif268, erg-1, NGF1A or Krox24). Male Japanese quail were allowed to copulate with a receptive female or to see her only and express appetitive sexual behavior. Control subjects were left undisturbed in their home cage. Brains were collected 90 min later and immunostained to detect and quantify the numbers of neurons expressing Zenk and aromatase at three rostro-caudal levels of the POM and in the BST. No differences among groups were detected in the total number of cells expressing these two proteins. However, the number of ARO-ir neurons simultaneously expressing Zenk was significantly higher in males allowed to copulate than in males allowed to see the female or in controls. The acute changes induced by copulation in aromatase activity measured in POM and BST are thus closely associated with an activation of ARO-ir neurons but there is no such evidence for the enzymatic changes induced by the simple view of a female. Additional experiments are needed to determine whether these enzymatic changes coincide with changes in another immediated early gene or occur without inducing a parallel change in gene transcription. Further analyses are also in progress to determine whether the activation of ARO-ir neurons by copulation is topographically organized.

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Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: R01 MH 50388

Title: Dopamine depletion in the medial preoptic nucleus and nucleus accumbens transiently impairs appetitive and consummatory sexual behaviors in male japanese quail

Authors: *O. IYILIKCI[1], J. BALTHAZART[2], G. F. BALL[1]
[1]Johns Hopkins Univ., Baltimore, MD; [2]GIGA Neurosciences, Univ. of Liege, Liege, Belgium

Abstract: There is ample evidence indicating that dopamine plays a critical role in the regulation of male sexual behavior. In particular, studies have demonstrated that dopamine release in the medial preoptic nucleus (POM) and nucleus accumbens (NAc) facilitates the occurrence of sexual behaviors. To elucidate further the role of dopamine in POM and NAc in the control of different aspects of sexual behavior in male quail and to investigate the source of these dopaminergic inputs, we stereotaxically injected a catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), into the POM and NAc. Control groups were injected with a sterile saline (0.9%) solution containing ascorbic acid (0.2%) into POM and NAc. To enhance the dopamine-selectivity of the lesions, an intraperitoneal injection of desipramine was administered thirty minutes prior to the delivery of 6-OHDA. Both appetitive and consummatory aspects of sexual behavior were monitored 1hr prior to surgery and at 5hr, 24hr, 1 week, and 2 weeks after the surgery. Brains were collected two weeks after the surgery on the last testing day. Appetitive behaviors were measured by the onset of the approach behavior and the amount of time spent in close proximity to a female quail behind Plexiglas glass door during the first two minutes of testing. Subsequently, the Plexiglas glass door was removed and the number of mounts and mount attempts of the male quail were documented to measure consummatory sexual behaviors. Animals exposed to 6-OHDA exhibited a rapid impairment in both aspects of sexual behavior and this impairment persisted for 5 hr and 24hr after 6-OHDA injections in both POM and NAc compared to control injections. However, there was complete recovery of these behaviors 1 week after surgery. Immunohistochemical analyses of the distribution of tyrosine hydroxylase immunoreactive (TH-ir) fibers in brain collected two weeks after injection demonstrated a decrease in TH-ir fibers density within the POM, indicating an impairment of dopaminergic inputs to this nucleus. Overall, this study demonstrates that dopaminergic innervation of the POM and NAc is necessary for the expression of appetitive and consummatory

sexual behavior in male quail. We are now following up on the behavioral restoration during the first week after injection to more precisely ascertain if this behavioral recovery is associated to re-growing of dopamine fibers or compensation of function by the remaining ones.

Disclosures: O. Iyilikci: None. **J. Balthazart:** None. **G.F. Ball:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 449.04/NN36

Topic: E.03. Behavioral Neuroendocrinology

Title: Arc expression in sexually-relevant brain areas following differing exposure to sexual stimuli and experience in male rats

Authors: *J. TURNER[1], E. A. HARVEY[2], T. HATTORI[2], R. G. WILL[2], D. J. TOBIANSKY[2], V. L. NUTSCH[2], J. M. DOMINUGEZ[2]
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Abstract: Coordinated activity in several brain areas is necessary for successful copulation, particularly in sexually naïve male rats. Some of these areas, including the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA), are primarily involved in the processing of sexually relevant olfactory cues. Others, such as the medial preoptic area (mPOA) of the hypothalamus, serve as multimodal integration centers that coordinate sexual behavior more generally. Previous studies have characterized activation of these brain areas following copulation and exposure to sexual stimuli by measuring expression of the immediate early gene c-Fos. Another immediate early gene, Arc, also serves as an indicator of neural activation. More specifically, Arc expression is indicative of plastic changes in activated dendritic synapses. We examined expression of the Arc protein product in the BNST, MeA, and mPOA to determine the effects of different sexual stimuli and prior experience on plastic changes in these areas. Groups of sexually experienced and sexually naïve male rats were placed in a copulation arena either alone, with an estrous female they could investigate but with which they could not copulate, or with an estrous female with which they were allowed to copulate. Here we discuss our findings and their implications for neural changes associated with sexual activity and sexual experience.

Disclosures: **J. Turner:** None. **E.A. Harvey:** None. **T. Hattori:** None. **R.G. Will:** None. **D.J. Tobiansky:** None. **V.L. Nutsch:** None. **J.M. Dominugez:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 449.05/OO1

Topic: E.03. Behavioral Neuroendocrinology

Title: Effects of opioid and dopamine agonists and antagonists on development of sexual motivation and sexual performance in sexually naïve male rats

Authors: M. BUENROSTRO-JAUREGUI[1,2], J. JUAREZ[2], *E. BARRIOS DE TOMASI[2]
[1]Inst. Vocacional Enrique Diaz de Leon, Guadalajara, Mexico; [2]Inst. Neurosci, Jalisco, Mexico

Abstract: It is known that previous sexual experience is not necessary for seeking or approaching (motivation) to sexual incentive. There is broad evidence that dopamine and opioid systems are involved in sexual behavior; however, it is unknown how these systems participate in the process of seeking and development of copulation in sexually naive subjects. The aim of this research was to determine how dopamine and opioid systems are involved in the development of the initial sexual intercourses, in both appetitive and consummatory phases. Sixty sexually naive adult male Wistar rats were randomly divided into six groups and exposed to different pharmacologic treatments: 2mg/kg bromocriptine (BRO), 5mg/kg methadone (MTD); 0.075mg/kg haloperidol (HAL), 3mg/kg naltrexone (NTX); saline(Sal) or 0.056 mg/kg alcohol (ETOH). All groups were provided with food and water ad libitum in a 12-12h light-dark cycle. At 85 days, motivation (proximity behavior) and sexual performance (mounts, intromissions and ejaculation) were recorded, which lasted up to 6 sessions separate for 72 hours between them. MTD decreased motivation, and sexual performance was practically abolished. All the other drugs showed no effect on the first session; however, NTX significantly decreased sexual motivation from the second to the sixth session, but consummatory components were not modified. BRO improved the performance of subjects in appetitive and consummatory parameters and 50% of subjects of this group ejaculated in the first interaction compared to none male in SAL group. HAL impaired both sexual motivation and sexual performance. Results suggest that MTD resembles the physiological state of sexual satiation, decreasing the appetitive behavior and suppressing copulation. NTX probably blocked the reinforcing/rewarding effects of endogenous opioids released during copulation from the first session, reducing sexual motivation

on following sessions. Taken together, these data suggest that the opioid system is essential for approaching behaviors, affecting the subsequent mating behavior, and its reinforcement. Paradoxically, overactivation of opioid system by MTD induce a severe inhibition of sexual behavior, resembling probably sexual satiety. On the other hand, dopaminergic system facilitated the development of consummatory behaviors in sexually naive subjects, possibly increasing the incentive value of the estrous female as a sexual stimulus.

Disclosures: M. Buenrostro-Jauregui: None. **J. Juarez:** None. **E. Barrios De Tomasi:** None.

Poster

449. Male Sexual Behavior

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Program#/Poster#: 449.06/OO2

Topic: E.03. Behavioral Neuroendocrinology

Support: Canadian Institutes for Health Research (CIHR)

Comisión Nacional de Investigación Científica y Tecnológica (CONICYT)

Canadian Bureau for International Education (CBIE)

Title: Conditioned ejaculatory preference by male rats for a somatosensory cue on a female rat

Authors: *G. R. QUINTANA ZUNINO, M. JACKSON, M. NASR, A. GUIZAR, J. TOMARO, A. ARGENTO, J. G. PFAUS
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Previously, we have shown that male rats form a conditioned ejaculatory preference (CEP) for females bearing an odor (e.g., almond) that was paired with the male's postejaculatory reward state, making the odor act as a discrete, partner-related cue. We have also shown that male rats wearing a rodent tethering jacket during their first sexual experiences lose sexual arousal and motivation if the jacket is removed prior to copulation, making the jacket act as a contextual cue for sexual arousal. Here we asked whether the rodent jacket could act as a discrete cue to establish a CEP. Two studies were conducted to evaluate this. In the first study, 12 sexually-naïve Long-Evans males underwent 14 multi-ejaculation trials with females wearing the rodent tethering jacket. On the final test, males were placed into an open field with two sexually receptive females, one with the jacket on and the other with the jacket off. A trend was found for more males to ejaculate first with females wearing the jacket relative to the females not wearing

the jacket. In the second study, 12 new males underwent an explicitly paired paradigm in which they were exposed sequentially to jacketed females that were sexually receptive and then unjacketed females that were not sexually receptive. The final open field test was run identically to the first. These males displayed a significantly higher number of ejaculations with the jacketed female, higher proportion of first ejaculation preference for the jacketed female, and a decrease in the ejaculation latency with the jacketed female. The brains were extracted from this group and analyzed for Fos-IR activation relative to males not trained to associate the jacket with copulation. This study demonstrates that a somatosensory cue previously used to establish sexual arousal as a contextual cue on the male can be used as a discrete, partner-based cue to establish a CEP for a particular female wearing the jacket.

Disclosures: G.R. Quintana Zunino: None. **M. Jackson:** None. **M. Nasr:** None. **A. Guizar:** None. **J. Tomaro:** None. **A. Argento:** None. **J.G. Pfaus:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 449.07/OO3

Topic: E.03. Behavioral Neuroendocrinology

Support: Canadian Institutes of Health Research (CIHR)

Title: Sign-tracking for sex: Individual differences in Pavlovian-conditioned approach behavior in male rats

Authors: *L. SPARKS, J. G. PFAUS
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Abstract: The pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US) results in individual differences in approach toward either the CS (sign-tracking; ST) or US (goal-tracking; GT) as evidenced by studies using food or drug reward. No study has yet examined ST or GT as a function of sexual reward. ***Objective***: The present study asked whether male rats might display ST or GT using the ejaculatory reward state as the US. ***Methods***: Sexually naïve, Long-Evans male rats received 11 Pavlovian conditioning trials in an individualized compartment of an open field chamber, where an orange cone CS (2-min/presentation) predicted the opportunity to copulate to two ejaculations in a separate compartment with a receptive female (US). During training and at test, behavior directed toward the CS (ST) and US (GT) was measured by time spent in an area centered around the CS or the door to the female compartment, respectively, in the absence and presence of the CS cue.

Results: On trials 1 and 6, rats did not display CS- or US-directed behavior; no differences were detected in time spent in the CS- and US- designated areas. On trial 12 however, ST was the predominant response of the males as measured by time spent in the CS-designated area compared to the US-designated area when the CS cue was present. There were also differences in copulatory behavior between ST and GT, such that the introduction of the CS during copulation in the compartment with the receptive female decreased the ejaculation latencies in males displaying ST, but not in males displaying GT. ***Conclusions***: Overall, these results demonstrate that conditioned cues acquire incentive motivational properties through Pavlovian conditioning using sexual reward, as we have shown previously for odor and somatosensory CSs. Similar to the literature on food or drug reward, the development of sexually-conditioned ST or GT behavior indicates the existence of individual differences in the attribution of incentive salience to a sexually-conditioned cue, where such cues might acquire greater motivational control over behavior than the sexual reward itself, as occurs in the expression of many sexual fetishes in humans. Funded by Canadian Institutes of Health Research (CIHR)

Disclosures: L. Sparks: None. **J.G. Pfaus:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

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Program#/Poster#: 449.08/OO4

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC RGPIN 312458-11 (DAM)

NSERC CGS D (ASG)

Title: Roles for neural and non-neural androgen receptors in the organization of masculine olfactory preference

Authors: *A. B. SWIFT-GALLANT[1], D. ALMEIDA[1], B. KRETSCHMER[1], F. RAMZAN[1,2], L. COOME[1,2], D. A. MONKS[1,2,3]
[1]Psychology, Univ. of Toronto Mississauga, Mississauga, ON, Canada; [2]Neurosci., [3]Cell and Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: Previous reports of conditional androgen insensitivity suggest that there may be a role for non-neural AR on the sexual differentiation of olfactory preference in male mice. To further

address this question, and to evaluate the role of androgens in the organization and activation of a male-typical olfactory preference, we have developed a loxP-based transgenic mouse, which overexpresses AR only when activated by Cre. We used this transgene to either overexpress AR globally in all tissues using a CMV-Cre driver or selectively in neural tissue using a Nestin-Cre driver. Female wildtype and transgenic animals were administered either a vehicle or testosterone on postnatal day 1 (PND1). Subsequently in adulthood, we tested these females 1) without further hormonal treatment, 2) following estrogen (E) treatment, and 3) following dihydrotestosterone (DHT) treatment, on an olfactory preference test where we simultaneously exposed animals to female-soiled, male-soiled and clean bedding. Adult E treatment activated a male-typical preference for female bedding in wildtype females treated on PND1 with testosterone, but not in PND1 vehicle treated wild types. Interestingly, adult E treatment resulted in a masculine odor preference in CMV-AR females regardless of PND1 treatment. In contrast, adult E treatment was not able to activate masculine odor preference in Nestin-AR females regardless of PND1 treatment, suggesting an inhibitory effect of neural AR on preference for female bedding. Combined adult E and DHT treatment did not activate masculine odor preference beyond E treatment alone. Together, these results suggest that a male-typical preference for female bedding can be organized by non-neural AR and is activated by estrogens in adulthood.

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Poster

449. Male Sexual Behavior

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 449.09/OO5

Topic: E.03. Behavioral Neuroendocrinology

Title: Effect of Bromocriptine in the reactivation of male copulatory behavior after sexual satiety with the same mating female

Authors: J. ROJAS-HERNÁNDEZ[1], *J. JUAREZ[2]
[1]Inst. de Neurociencias Univ. de Guadalajara, Guadalajara, Mexico; [2]Inst. Neuroci, Univ. Guadalajara, Guadalajara, Jalisco, Mexico

Abstract: The male sexual satiety is the cessation of copulatory behavior following several ejaculations, causing a decrease in sexual motivation. This phenomenon is believed to be

responsible for the sexual inhibition, which, in turn, has been associated with a decrease in dopamine levels, and an increase in prolactin levels. It has been described that the spontaneous recovery of copulatory behavior begins at least 72 hours after sexual satiety is reached. Several authors report that it is possible to reverse sexual satiation 24 hours after established by pharmacological treatments that activate the dopamine system. It has also been reported that if a sexually satiated male is exposed to a new receptive female different to that with which the sexual satiety was reached, sexual intercourse is reactivated immediately, a phenomenon known as "Coolidge effect," which is caused by a renewal of sexual motivation, accompanied by an increase in dopamine release. On this basis, the aim of this work was to explore whether dopaminergic activation by bromocriptine (BrCr), could resume copulatory behavior with the same female immediately after sexual satiety was reached. For this, male rats were divided into three groups and each was exposed to one of three conditions: 1) administration of 2 mg/Kg sc of BrCr and exposure to the same female with whom reached sexual satiety; 2) administration of a 0.3 ml sc of vehicle solution and exposure to the same female with sexual satiety was reached, and 3) exposure to a new receptive female after sexual satiety. Results showed that BrCr reactivated the copulatory capability in sexually satiated males with the same mating female, in a similar way as observed in males without treatment and exposed to a new receptive female. Contrarily, several males with vehicle treatment reactivated copulation, but none showed ejaculation with the same mating female after sexual satiation. The reversal of sexual satiety state in males treated with BrCr could be explained by stimulation of D2 receptors, localized in the Nucleus Accumbens and medial preoptic area, promoting a renewal in sexual motivation subsequent to satiety, in a sufficient level to allow the reactivation of copulation with the same mating female. Stimulation of D2 receptors in dorsal striatum, paraventricular nucleus and spinal autonomic nuclei, could also play an important role in the resumption of the copulatory behavior. Moreover, a negative relationship between prolactin and dopamine has been described in the course of sexual satiety phenomenon; therefore it is possible that a decrease in serum prolactin levels caused by BrCr, may also contribute to the phenomenon of reversal of sexual satiation.

Disclosures: **J. Rojas-Hernández:** None. **J. Juarez:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

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Program#/Poster#: 449.10/OO6

Topic: E.03. Behavioral Neuroendocrinology

Title: GABAergic transmission participates in the maintenance of the sexual inhibition that follows copulation to exhaustion

Authors: *G. RODRIGUEZ-MANZO, A. CANSECO-ALBA
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Abstract: It has been demonstrated that GABAergic transmission plays an important role in the control of the refractory period that follows ejaculation in male rats, the post-ejaculatory interval (PEI). An increase in rat cerebrospinal fluid GABA levels occurs immediately after ejaculation and GABA-A receptor antagonism within the medial preoptic area (mPOA), but not in other brain regions, produces an important reduction in PEI duration, suggesting that the refractory period is a consequence of an increased GABAergic transmission within the mPOA. Sexual exhaustion consists of a long lasting sexual behavior inhibition that appears as a consequence of repeated ejaculation. Copulation to satiation is characterized by the gradual exponential increase in the duration of the PEI after each successive ejaculation, until the male fails to resume copulation within 90 min after the last ejaculation. Thus, GABAergic transmission in the mPOA could be thought to play a role in the development and maintenance of the sexual inhibitory state that characterizes sexual satiation. However, this hypothesis could not be confirmed, as this sexual inhibitory state was not reversed by antagonizing GABA-A receptors in the mPOA. The pharmacological analysis of sexual exhaustion has increasingly evidenced the involvement of the mesolimbic system in the induction and maintenance of its long lasting sexual inhibition. GABAergic interneurons of the mesolimbic system participate in the control of the functioning of this brain circuit. On these bases we decided to test the hypothesis that GABAergic transmission at other brain regions could play a role in sexual satiation. To this aim, independent groups of sexually exhausted male rats were systemically injected with low doses of the GABA-A receptor antagonist bicuculline (0.001-0.3 mg/kg, i.p.), administered 24 h after copulation to satiation and tested for sexual behavior. Results showed that almost all bicuculline doses tested significantly increased the percentage of satiated rats copulating to ejaculation and that doses ranging from 0.01 to 0.3 mg/kg reversed sexual exhaustion in almost 80% of the subjects. These same bicuculline doses almost lacked an effect on the copulatory behavior of sexually experienced rats. It is concluded that GABAergic transmission participates in the maintenance of the sexual inhibition that follows copulation to satiation by acting at brain areas other than the mPOA.

Disclosures: G. Rodriguez-Manzo: None. **A. Canseco-Alba:** None.

Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant MH040826 to EMH

Wabash College

Title: A nitric oxide promoter in the medial preoptic area facilitates copulation in adult male rats

Authors: B. WISE[1], *T. M. AUBELE-FUTCH[2], E. M. HULL[3]
[2]Psychology, [1]Wabash Col., Crawfordsville, IN; [3]Florida State Univ., Tallahassee, FL

Abstract: Dopamine (DA) in the medial preoptic area (MPOA) is crucial in order for adult male rats to perform their entire range of copulatory behaviors. Previous research has shown that the nitric oxide (NO)-cGMP pathway modulates and facilitates MPOA DA levels, and that administration of a NO synthesis inhibitor directly to the MPOA inhibits both basal and female-stimulated MPOA DA release, stimulus sensitization, and copulatory ability. Thus, we hypothesized that administration of a NO promoter, sodium nitroprusside (SNP), could mimic the facilitative effects of stimulus sensitization on copulatory ability. In naïve male Long-Evans Blue Spruce rats, SNP or saline was microinjected directly to the MPOA every other day for a total of 7 administrations. Other naïve male rats were exposed to receptive females placed over their cages on the same schedule, and a third cohort received IP injections of SNP on the same schedule. Those receiving SNP or saline did not receive exposure to females or female odors until copulatory testing. In a drug-free test after the 7th administration of SNP, saline, or exposure to a receptive female, copulatory behaviors (mounts, intromissions, and ejaculations) were scored in a single copulatory session lasting thirty minutes from the first intromission. Animals that received SNP directly to the MPOA showed facilitation of some, but not all, sexual behaviors, including ejaculation frequency and intromission-to-mount ratios over discrete ejaculatory series, similarly to those males that were stimulus-sensitized via exposure to receptive female rats. Animals receiving SNP regardless of the route of administration also showed lower post-ejaculatory intervals, similar to female-exposed animals. Thus, a NO promoter administered to the MPOA can mimic some facilitative aspects of stimulus sensitization in adult male rats.

Disclosures: B. Wise: None. **T.M. Aubele-Futch:** None. **E.M. Hull:** None.

Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: OLLI (Osher Lifelong Learning Institue) research scholarship to CLR

Title: Behavioral and neuroendocrine effects of carbon monoxide in the anterior hypothalamus

Authors: *C. L. ROBISON[1], T. CIRINO[2], O. K. HERNANDEZ[2], E. M. HULL[2]
[2]Psychology, [1]Florida State Univ., Tallahassee, FL

Abstract: We have previously reported that carbon monoxide (CO) signaling in the medial preoptic area (MPOA) has behavioral effects similar to those observed after the administration of nitric oxide (NO), which is generally pro-copulatory. Here, we expand upon this research to demonstrate that the administration of hemin, a CO donor, and a heme oxygenase-2 (HO2, the primary CO-producing enzyme in the brain) mRNA knockdown have opposite effects on copulatory and anxiety-like behaviors in the rat, in line with our previous reports that SnMP-IX (a HO2 inhibitor) and CORM-2 (a CO-releasing molecule) inhibit and facilitate copulatory behaviors, respectively. Since CO may affect multiple molecular pathways, only some of which are shared with NO, we examine whether the manipulation of two putative downstream pathways, the cGMP (cyclic GMP, a second messenger important for neurotransmitter release from the axon terminal) and PGE-2 (prostaglandin E2, an “anti-inflammatory” prostaglandin with a number of known effects in the CNS) systems. Since HO2 is expressed at moderate levels in the MPOA and expression increases in a site-specific manner after multiple sexual experiences, our results to date indicate that CO is a potentially significant natural regulator of MPOA function. Furthermore, we also examine the effects of CO on end-point hormones of the HPA and HPG axes, both of which are influenced by MPOA activity and affected by CO administration in *in vitro* hypothalamic models but have not previously been shown to be regulated by CO manipulation *in vivo*.

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Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: DCBS-UAMI-2010

Title: Neurobehavioral effects of cadmium after exposition until puberty in adult male rats

Authors: *A. MARCELA[1], T. MENDOZA-MENDOZA[1], J. HERNÁNDEZ-RODRIGUEZ[1], O. LIMÓN-MORALES[1], R. M. VIGUERAS-VILLASEÑOR[2], H. BONILLA-JAIME[1], S. MONTES-LÓPEZ[3], P. DURAN[4]
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Abstract: The study of neurobehavioral effects, including sexual behavior, exposure to cadmium (Cd+2) is relevant, because human are exposed to significant concentrations of this metal. The Cd+2 is a neurotoxic interest, especially when exposure occurs during the period of early development and puberty, since it affects liver, kidneys, lungs and heart, but also alters those CNS structures which regulate reproductive functions on reproductive organs such as the testis, causing damage to the seminiferous tubules and a decrease in testosterone levels. The incidence of neurodevelopmental toxicity and the impact of this on sexual behavior depends of the largely exposure to this metal, so it is important to study the effect of Cd+2 on behavior male sex, and in his motivational execution stages for this aim. The newborn male rats were used; which were randomly assigned to a mother, height pups by one mother. They were kept in a light-dark cycle reversed 12:00 to 12:00, the pups were injected ip with a solution of cadmium chloride at doses of 0.5 and 1 mg kg in a volume of 600 ul injection and other were injected with saline (control group) (600µl), vehicle where cadmium chloride dissolved. The injections were daily up to 56 days of postnatal life, at which time the rats have already gone through the implementation and testing puberty. The sexual incentive motivation test were performed at 120 days of age, immediately after males exposed to Cd+2 at 0.5 and 1 mg/kg and controls were sacrificed by decapitation for obtaining serum and subsequent measurement of testosterone by RIA. In addition, plasma was obtained meanwhile, hypothalamus, prefrontal cortex, olfactory bulb, testis, seminal vesicle, epididimus were dissected and analyzed by atomic absorbance spectrophotometry in order to quantify Cd+2 . Regarding to sexual behavior (CS) and the sexual incentive motivation tests, Cd+2 groups, showed a decrease in the copulatory eficency as well as in the incentive sexual motivation and a decrease in the serum testosterone level when compared to controls. Therefore, we conclude that exposure to Cd+2 yields to dose dependent neurobehavioral function deficit, as well as to an altered decrease in testosterone.

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Poster

449. Male Sexual Behavior

Location: Halls A-C

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Program#/Poster#: 449.14/OO10

Topic: E.03. Behavioral Neuroendocrinology

Support: Brinkman Foundation Grant

Title: The effects of acute prenatal exposure to valproic acid and environmental enrichment on anxiety and sociosexual behaviors in male rats

Authors: *S. M. HARDING, J. A. CAPUTO, H. I. HORVATH
Psychology Dept, Fairfield Univ., FAIRFIELD, CT

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that occurs more often in boys and is characterized by social impairments and deficits in communication. Recent research in rodents suggests that *in utero* exposure to a high dose of valproic acid (VPA) can induce ASD-like behavioral changes in male offspring, including increased anxiety, heightened sensitivity to sensory stimulation, and aberrant social behaviors; although social behavior tests have largely been confined to same sex (male-male) interactions. The present study was conducted to examine the effects of an acute moderate dose of VPA administered prenatally on (1) general anxiety and (2) social behaviors with females (sociosexual behaviors) in adulthood. Additionally we evaluated if environmental enrichment (EE) after weaning could prevent ASD-like phenotypes. Pregnant Long Evans rats were injected i.p. with VPA (350mg/kg; n=2) or saline (n=2) on gestational day 12.5. Male pups were then assigned to two housing conditions from postnatal day (P)22-P36: EE or standard housing, resulting in four groups: VPA-enriched, VPA-standard, Saline-enriched, and Saline-standard. After P36, males were pair-housed in standard cages for the remainder of the study. Anxiety was assessed with the elevated plus maze (EPM) and the emergence test, and sociosexual behaviors were assessed with tests for copulation, partner preference and ultrasonic vocalizations. Preliminary data analysis suggests that moderate VPA exposure enhances anxiety. In the EPM test, VPA-exposed males spend less time in the open arms (p=.021) and make fewer entries into open arms (p=.02) compared to Saline-exposed males. In the emergence test, VPA-standard males took longer to leave a hide

box than VPA-enriched males ($p<.08$), suggesting that EE may partially counteract the detrimental effects of VPA on anxiety. Surprisingly, neither VPA exposure nor EE seem to impair social interactions with females in copulation tests or partner preference tests. However, EE may increase the frequency of ultrasonic vocalizations, independent of prenatal conditions. These results suggest that while a moderate dose of VPA during development may be sufficient to induce anxiety in male rats, that anxiety may not extend to abnormal social interactions with females. Follow up studies will examine if these behavioral patterns persist with higher doses of VPA.

Disclosures: S.M. Harding: None. **J.A. Caputo:** None. **H.I. Horvath:** None.

Poster

449. Male Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC

Title: Prosexual effects of cabergoline, a unique ergot derivative, in male rats

Authors: *R. A. ANTONIE[1], N. DEVOTO[2], P. DORSA[3], S. KIM[4], J. PFAUS[2]
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Abstract: Cabergoline is a semi-synthetic ergot derivative with a unique pharmacological profile. Through its long-acting actions on dopamine (DA) D2 receptors, it is able to suppress the release of prolactin selectively from trophic cells in the anterior pituitary, and it is currently approved for the treatment of hyperprolactinemia and prolactinomas. However, it also possesses binding affinity for D3, D4, 5-HT1A, 5-HT2A, 2B, and 2C, and α2B receptors, and low-to-moderate affinity for D1 and 5-HT7 receptors. It acts as an antagonist at α2B and 5-HT7 receptors. Cabergoline is prescribed off-label to treat the lack of sexual desire and orgasm associated with hyperprolactinemia and similar effects of chronic SSRI medication. Here we asked whether cabergoline on its own would alter the sexual behavior of sexually experienced male rats. Male Long-Evans rats (N=40) were given 5 multiejaculatory sexual experiences at 4-day intervals in bilevel chambers prior to daily oral administration by gavage of one of four doses of cabergoline (0, 0.003, 0.015, or 0.3 mg/ml) for 68 days. During this period, males

received an additional 16 copulation tests in bilevel chambers at 4-day intervals. All tests were 30 min in duration and conducted during the middle third of the rat's dark cycle. The medium and high doses of cabergoline produced a dramatic and significant increase in anticipatory level changes, intromissions, and ejaculations, and a trend toward a significant decrease in the postejaculatory interval, compared to the low dose and control. These effects were observed early and persisted throughout the treatment regimen, suggesting a lack of tolerance. To examine the pattern of brain activation by cabergoline alone, rats in each group were injected with their dose of cabergoline or control alone for an additional 4 days after the last copulatory trial and then given an overdose of sodium pentobarbital 4 hrs after the last cabergoline (when they would have been tested), perfused with saline and paraformaldehyde, and their brains extracted and prepared for immunohistochemistry for Fos protein. Significant numbers of Fos positive cells were found in the nucleus accumbens, medial preoptic area, and ventromedial hypothalamus of rats given the moderate to high doses. These results show that cabergoline alone has prosexual effects in sexually experienced male rats and that the drug activates key regions of the brain that are excitatory for appetitive and consummatory aspects of sexual behavior.

Disclosures: R.A. Antonie: None. **N. Devoto:** None. **P. Dorsa:** None. **S. Kim:** None. **J. Pfaus:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 449.16/OO12

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01-MH068764

Title: Pubertal testosterone regulates the induction of ΔFosB in the infralimbic cortex to program sexual proficiency in male Syrian hamsters

Authors: *K. C. DE LORME[1,2], A. J. ROBISON[2], C. L. SISK[2]
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Abstract: Social proficiency is acquired through the ability to make behavioral adaptations as one learns from social experience; it involves both behavioral flexibility and inhibition of behaviors inappropriate for a specific social context. The acquisition of social proficiency during

adolescence is particularly advantageous for successful social interactions in adulthood. Here we investigate the contribution of testosterone and ΔFosB to the adolescent maturation of social proficiency in male-female social interactions. Testosterone, acting during puberty, organizes neural circuits underlying social behaviors; ΔFosB is a transcription factor linked to experience-dependent neural plasticity. To determine whether pubertal testosterone organizes circuits underlying social proficiency, we compared behavioral adaptations to sexual experience in male Syrian hamsters that were deprived of testosterone during puberty (prepubertal castration; NoT@P) to those of males deprived of testosterone for an equivalent period of time in adulthood (postpubertal castration; T@P). All males were given testosterone replacement in adulthood for two weeks before sexual behavior testing, in which each male was allowed to interact with a receptive female until the male achieved the specific sexual behavior criteria for that trial (e.g., one ejaculation) once per week for five weeks. We first tested the hypothesis that the adolescent gain in sexual proficiency depends on organizational effects of testosterone. We found that NoT@P males continued to show high rates of ectopic (mis-directed) mounts with sexual experience, whereas T@P males had overall lower rates of ectopic mounts compared to NoT@P males and a decrease after sexual experience. Ectopic mounting is often displayed by sexually naïve male hamsters, but with sexual experience, it normally decreases to low levels, enhancing sexual proficiency. These data suggest that pubertal testosterone programs the ability to adapt behaviors via inhibition in a social context-dependent manner. We then tested whether this effect requires regulation of ΔFosB in specific brain regions. Using immunohistochemistry, we found that ΔFosB was induced in the infralimbic cortex (IL) in sexually experienced T@P, but not experienced NoT@P, males. Furthermore, over-expressing ΔFosB via adeno-associated viral vectors in the IL of NoT@P males prior to sexual behavior testing was sufficient to reverse the high rates of ectopic mounting. Taken together, these data provide evidence that the ability to inhibit inappropriate behavior during a sexual encounter is organized by pubertal testosterone through the regulation of ΔFosB in the IL.

Disclosures: **K.C. De Lorme:** None. **A.J. Robison:** None. **C.L. Sisk:** None.

Poster

449. Male Sexual Behavior

Location: Halls A-C

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Program#/Poster#: 449.17/OO13

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R00HD056041-05

Title: Dendritic morphology of medial preoptic neurons of ovariectomized female hybrid B6D2F1 mice that demonstrate steroid-independent male-typical reproductive behavior

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Abstract: In most rodent models studied in the lab, male sexual behavior (MSB) is highly dependent upon gonadal steroids; however, many other mammalian species, including humans, continue to demonstrate MSB in the absence of gonadal steroids, yet the mechanisms underlying this phenomenon are not well understood. One of the few exceptions to the rodent model is the male B6D2F1 hybrid mice, in which ~30% of the males display MSB up to 26 weeks after castration (herein, referred to as "maters"). Mirroring their male counterparts, our laboratory has shown that the females of this hybrid strain also demonstrate male-typical sexual behavior (mounting and pelvic thrusting), long after ovariectomy. Normally, male-typical sexual behavior is rarely observed in ovary-intact or ovariectomized female rodents. Thus, this hybrid strain provides an excellent model to utilize to help unravel the complex molecular and neuronal mechanisms that contribute to individual variation in MSB, sexual differentiation, as well as furthering our understanding of the evolution by which behaviors have become emancipated from hormonal dependence. Recently, our lab has shown that steroid-independent MSB is correlated with differences in dendritic architecture on neurons in a brain region that is integral for the expression of MSB. Specifically, hybrid male maters exhibited greater dendritic spine density of medial preoptic neurons relative to non-maters. In the present study, we sought to determine whether the neuroplasticity that is correlated with steroid-independent MSB in the males, was present in the females by assessing the dendritic morphology of medial preoptic neurons in Golgi-impregnated brains of ovariectomized B6D2F1 hybrid females. Interestingly, our preliminary results showed that females that demonstrated male-typical sexual behaviors did not exhibit greater dendritic spine density of medial preoptic neurons relative to those that did not, indicating a sex difference between the dendritic architecture between male "maters" and ovariectomized B6D2F1 female hybrids that displayed male-typical reproductive behavior.

Disclosures: J. Park: None. **P. Bharadwaj:** None. **S. Venu:** None.

Poster

450. Defensive Behavior and Aggression

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Program#/Poster#: 450.01/OO14

Topic: E.03. Behavioral Neuroendocrinology

Support: Z01-MH-002498-24

Title: Raphe serotonin neuron-specific oxytocin receptor knockout reduces aggression but not anxiety-like behavior in male mice only

Authors: *S. WILLIAMS[1], J. PAGANI[2], J. SONG[2], É. MEZEY,[3], J. SENERTH[2], Z. CUI[4], M. H. BAUMANN[5], W. S. YOUNG[2]
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Abstract: Serotonin and oxytocin influence aggressive and anxiety-like behaviors, though it is unclear how the two may interact. That the oxytocin receptor is expressed in the serotonergic raphe nuclei suggests a mechanism by which the two neurotransmitters may cooperatively influence behavior. We hypothesized that oxytocin acts on raphe neurons to influence serotonergically-mediated anxiety-like, aggression and parental care behaviors. We eliminated expression of the oxytocin receptor in raphe neurons by crossing mice expressing Cre recombinase under control of the serotonin transporter promoter (Slc6a4) with our conditional oxytocin receptor knockout line. The knockout mice generated by this cross are normal across a range of behavioral measures: there are no effects for either sex on locomotion in an open-field, olfactory habituation/dishabituation or, surprisingly, anxiety-like behaviors in the elevated O and plus mazes. There was a profound deficit in male aggression: only one of 11 raphe oxytocin receptor knockouts showed any aggressive behavior, compared to eight of 11 wildtypes. In contrast, female knockouts displayed no deficits in maternal aggression. Our results show that oxytocin, via its effects on raphe neurons, is a key regulator of resident-intruder aggression in males but not maternal aggression. Furthermore, this reduction in male aggression is quite different from the effects reported previously after forebrain or total elimination of oxytocin receptors. Finally, we conclude that when constitutively eliminated, oxytocin receptors expressed by serotonin cells do not contribute to anxiety-like behaviors or maternal care.

Disclosures: S. Williams: None. **J. Pagani:** None. **J. Song:** None. **É. Mezey,:** None. **J. Senerth:** None. **Z. Cui:** None. **M.H. Baumann:** None. **W.S. Young:** None.

Poster

450. Defensive Behavior and Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH grant MH86542

NIH grant P51 OD11092

Title: Aromatase co-localizes in serotonin neurons and is not regulated, but androgen receptors (AR) do not co-localize in serotonin neurons and are regulated in male macaques

Authors: *C. L. BETHEA, A. KIM, K. PHU
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Abstract: It has been suggested that androgens act to decrease serotonin, which in turn underlies aggression in males. However, the mechanism is not well explored. We found that androgen treatment ± aromatase inhibition increased serotonin-related gene expression in male macaques, which departs from suppositions based upon measurement of serotonin metabolites in CSF. This study determines whether androgen-stimulated tryptophan hydroxylase2 (TPH2) and serotonin transporter (SERT) gene expression is directly mediated by AR, and it examines aromatase expression in serotonin neurons of the dorsal raphe nucleus (DRN). Aromatase and AR were detected with immunohistochemistry in serial sections through the dorsal raphe of castrated male macaques treated for 3 months with testosterone (T), placebo, dihydrotestosterone (DHT) + ATD (aromatase inhibitor) or flutamide (FLUT) + ATD (n=5/group). Aromatase was detected in the large serotonin neurons of the DRN, but it was not affected by treatment. Across the groups, average aromatase positive pixel area (x10e3) equaled 14.9±1.8, 16.4±1.0, 18.7±0.9, 15.6±1.0 ($p=0.27$) and aromatase+ cells equaled 500±56, 561±25, 621±31 and 504±36 ($p=0.18$). Double immunohistochemistry for AR [DAB detection] and TPH [blue chromagen detection] found numerous AR+ neurons in the DRN, but they were separate from the TPH-positive serotonin neurons. In single-labeled sections, average AR+ pixel area (x10e3) equaled 2.3±0.1, 1.8±0.1, 2.6±0.3, 1.4±0.1 ($p<0.0009$) and AR+ cell number equaled 455±31, 318±25, 455±48 and 263±17 ($p < 0.001$, respectively). Together the data indicate that aromatase was expressed in serotonin neurons (and others), but it was not affected by treatment. Conversely, AR was not expressed in serotonin neurons, but it was significantly increased by androgen administration, which is consistent with early reports on hypothalamic AR expression. The data strongly indicate that androgens increase serotonin-related gene expression by acting in other AR+ neurons. We hypothesize that the AR+ neurons in the DRN are stimulatory to serotonin neurons. Inhibition of aromatase activity did not alter detection of aromatase. Altogether, we have no evidence to support the notion that androgens decrease serotonin. Supported by NIH grants MH86542 to CLB and P51 O11092 for support of ONPRC.

Disclosures: **C.L. Bethea:** None. **A. Kim:** None. **K. Phu:** None.

Poster

450. Defensive Behavior and Aggression

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Program#/Poster#: 450.03/OO16

Topic: E.03. Behavioral Neuroendocrinology

Support: CNPq Universal Grant

INCT-MT Grant

Title: Behavioral profiles and immunologic correlates of Wistar rats exposed to intermittent social defeat stress protocol

Authors: ***M. F. VASCONCELOS**[1], C. P. CHATAIN[1], B. L. GUAHYBA[1], S. W. GEHRES[1], F. P. KAPCZINSKI[1], K. A. MICZEK[2], R. M. M. DE ALMEIDA[1]
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Abstract: Stress response is essentially mediated by Hypothalamus-Pituitary-Adrenal (HPA) and Sympathetic-Adreno-Medullary (SAM) axes, which act through the release of corticosteroids and catecholamines, respectively. In a broad sense, humoral and cellular immune responses are activated by catecholamines and inhibited by corticosteroids. Neurobiological basis of individual variability to stress can be related to distinct mutual activation of these axes. More aggressive responses are characterized by higher SAM and lower HPA-axes activation, whereas the opposite can be observed in less aggressive responding individuals. Thus, exposition to several physiological stressors strongly affects immune responses. Even though psychosocial stress is also known to activate HPA and SAM axes, the link between social defeat and immune responses is not clear yet. Therefore, our study aims to provide insights to a better understanding of the influence of social stress on immunological responses, as well as elucidate individual variability to stress responses. Social defeat stress was induced on male Wistar rats (n =20) by short confrontations with an aggressive resident every third day for 10 days. Control animals (n = 21) were handled and exposed to new home cages following the same schedule. Behaviors were analyzed and grouped into aggressive offensive, defensive and social exploration scores. Cytokines IL-10 and TNF-α levels were measured in serum samples using flow cytometry technique 24h after the last defeat session. Measurements of corticosterone and IGF-I are in progress. Stressed animals did not present significant differences in weight gain and cytokine

levels after 10 days of psychosocial stress. However, behavior analysis indicated the presence of two different groups of individual behavioral profiles of stress reactivity at the first defeat encounter. These profiles were characterized by higher or lower scores of aggressive offensive and defensive behaviors. No differences were observed between groups in respect to social exploration scores. Surprisingly, at the fourth defeat session the animals did not differ on behavioral scores. This result indicates a trend towards homogeneity in individual differences as an effect of persistence of psychosocial stress exposure. This study provides new evidence of social stress equalizing effects on individual reactivity to environmental stimuli. There were no evidence establishing a relationship between the behavioral and physiological phenotypes of coping strategies in response to stress and immune reactivity.

Disclosures: **M.F. Vasconcelos:** None. **C.P. Chatain:** None. **B.L. Guahyba:** None. **S.W. Gehres:** None. **F.P. Kapczinski:** None. **K.A. Miczek:** None. **R.M.M. de Almeida:** None.

Poster

450. Defensive Behavior and Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: CAPES

CNPq

FAPEMIG

Title: Reduction of the vesicular acetylcholine transporter expression exacerbates aggressive behavior and the activation of vasopressinergic hypothalamic neurons

Authors: ***G. S. PEREIRA**, V. E. M. OLIVEIRA, L. M. PEREIRA, M. D. POLETINI, M. F. D. MORAES
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Abstract: The cholinergic system modulates the pro-aggressive hormone synthesis, vasopressin (AVP). However, little is known about the acetylcholine (ACh) role on agonistic behavior. We hypothesized that reducing the cholinergic tonus may increase the vasopressin level and generates an exacerbated aggressive behavior in mice. We used knockdown mice for the vesicular acetylcholine transporter (KDHET) as a model of reduced cholinergic tonus. Male

mice were submitted to resident intruder test (RI), which consisted in isolating mice in a standard box during 2h and after confront it with an intruder. We measured during 10 minutes the time and frequency of agonistic behavior. KDHET mice present higher attack and tail rattle number as well as spent more time performing agonistic behavior. Further, the latency for the first attack was reduced in the KDHET. To verify if restoring the ACh levels would decrease the aggressive behavior, we administrated galantamine (GAL) 30 min before exposing animals to RI. KDHET mice treated with GAL attack less and spent less time in the agonistic behavior compared to saline treated mice. Next, we investigated the expression of AVP by immunohistochemistry. We observed no difference between genotypes regarding basal AVP level in the following hypothalamic nuclei: paraventricular (PVN), supra-optic (SON), anterior (AH) and lateral (LH). Thus, we decided to verify the AVP expression after exposing animals to RI. Interestingly, there was an increase in AVP expression after RI in all areas, but only in KDHET mice. We then decided to verify the activation of these neural substrates after RI by quantifying the c-Fos expression. PVN and AH were activated by RI in both genotypes. However, LH was more activated in the mutant mice. Finally, we quantified the AVP neurons activated after RI. The activation of AVP neurons from PVN and LH was higher in mutant mice exposed to RI. Our results showed that VAChT knockdown exacerbated aggressiveness in mice, which was prevented by the acetylcholinesterase inhibitor (GAL), indicating the cholinergic system as a direct modulator of aggressiveness. We also found a genotype-specific increase in AVP expression after RI in all areas quantified, which is in accordance with the exacerbated aggressiveness observed in mutant mice. Furthermore, the mutant mice showed an over activation of LH after RI. Finally, the proportion of AVP p ositive neurons after RI differs between genotypes. AVP neurons from PVN and LH are more activated in KDHET mice, than in WT. Taken together, our results suggest that the reduction in the ACh release increases aggressive behavior, probably by modulating the vasopressinergic system.

Disclosures: **G.S. Pereira:** None. **V.E.M. Oliveira:** None. **L.M. Pereira:** None. **M.D. Poletini:** None. **M.F.D. Moraes:** None.

Poster

450. Defensive Behavior and Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant DA010547-14

Title: Microiontophoretic application of vasopressin, serotonin, and dopamine ligands affect the electrophysiological activity of latero-anterior hypothalamic neurons in anabolic steroid-treated hamsters

Authors: *R. W. SIKES[1], T. R. MORRISON[2], R. H. MELLONI, Jr.[2]
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Abstract: Data from Syrian hamsters exposed to moderate doses of commonly abused anabolic/androgenic steroids (AAS) during adolescence consistently show that AAS exposure increases the display of aggressive behavior in early adulthood. Immunocytochemistry data as well as behavioral pharmacological data indicates that enhanced aggression correlates with alterations in various neurochemical systems within the latero-anterior hypothalamus (LAH). Most notably, LAH vasopressin (AVP) afferents are enhanced in aggressive AAS-exposed hamsters while 5HT afferents are decreased. These anatomical alterations are mirrored by behavioral data that show that systemic 5HT receptor agonists as well as locally applied AVP receptor antagonists within the LAH block aggressive behavior in AAS-treated animals. Recent data show that AAS enhances the presence of dopamine (DA) neurons within the nucleus circularis which possesses neurons that project into the LAH. Accordingly, microinjection of the DA D2 antagonist eticlopride into the LAH decreases aggressive behavior in AAS treated animals. This DA-evoked decrease is reversed by AVP microinjection, suggesting that DA modulates AVP release in the LAH. Despite the behavioral effects of compounds that target DA-, AVP-, and 5HT sensitive cells within the LAH after AAS exposure, little is known about the physiology of these neurons. To characterize how these cells interact at the functional level, we measured the electrophysiological response to microiontophoretically applied 5HT, eticlopride, and AVP in the anesthetized aggressive AAS-treated hamster during adolescent development. Preliminary data have revealed that the majority of cells tested respond to at least one of the three compounds. AVP primarily increased spontaneous activity, while 5HT and eticlopride primarily decreased spontaneous neuronal activity. When we examined the conditional effects of different compounds on the activity of a single cell we found that more than half of all AVP-responsive cells were also affected by both 5ht and eticlopride. These effects were by and large not present in vehicle treated animals. These data shed further light on how developmental exposure to AAS affects the physiology of the neural substrates of aggressive behavior. These data also expand our understanding of how adolescent AAS-induced alteration of various proteins affect how neurons within the LAH interact at the functional level.

Disclosures: R.W. Sikes: None. **T.R. Morrison:** None. **R.H. Melloni:** None.

Poster

450. Defensive Behavior and Aggression

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 450.06/OO19

Topic: E.03. Behavioral Neuroendocrinology

Title: Moderate anabolic/androgenic steroid use during adolescence and adulthood differentially modulate aggression and anxiety in hamsters

Authors: *R. H. MELLONI, Jr, L. A. RICCI, T. R. MORRISON
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Abstract: Anabolic/androgenic steroid (AAS) use remains high in both teens and adults in the U.S. and worldwide despite data that indicate that AAS use is associated with a higher incidence of aggression and anxiety. In previous studies, we showed that pubertal male Syrian hamsters (Mesocricetus auratus) exposed to moderate doses of commonly used AAS display a high level of aggression during AAS exposure and anxiety during AAS withdrawal, and that these behavioral changes occur alongside a predictive relationship over time. In particular, the decrease in aggression from exposure to withdrawal predicts an increase in anxiety during this time span. The current study investigates whether exposure to moderate doses of AAS during adulthood have similar effects on the relationship between aggression and anxiety. To investigate this, pubertal and adult male hamsters were administered a moderate dose AAS cocktail (5.0mg/kg/day x 30days) during adolescence (P27-56) or young adulthood (P65-P94) then tested for aggression and anxiety during AAS exposure (i.e., on P57 or P95) and then during AAS withdrawal (i.e., 30 days later on P77 or P115, respectively). As reported previously, adolescent exposure to moderate dose AAS increased aggressive responding during the AAS exposure period and anxiety-like responding during AAS withdrawal. In contrast, neither behaviors were similarly influenced by adult exposure to moderate doses of AAS. Unlike adolescent animals, adult AAS exposure to moderate dose AAS produced no difference in aggressive responding during AAS exposure (P95) or AAS withdrawal (P115) compared to controls. However, while moderate dose AAS exposure during adulthood produced no difference in anxiety-like responding during AAS exposure (on P95), adult hamsters administered AAS were far less anxious than vehicle control animals during extended periods of AAS withdrawal (on P115) - suggesting that adult exposure to AAS is anxiolytic. Together these data suggest that the aggression and anxiety provoking influence of moderate-dose AAS arises only when the exposure period to AAS occurs early (e.g., during adolescence) representing a developmental phenomenon. Moreover, these data also suggest that adult exposure to moderate doses of AAS may in fact be anxiolytic over the long term.

Disclosures: R.H. Melloni: None. **L.A. Ricci:** None. **T.R. Morrison:** None.

Poster

450. Defensive Behavior and Aggression

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 450.07/OO20

Topic: E.03. Behavioral Neuroendocrinology

Support: Call for PhD projects 2014 DCN

Title: The role of the 5-HT_{1A} receptor in pathological aggression

Authors: *D. PEETERS[1], H. TOP[2], R.-J. VERKES[3], J. HOMBERG[2]
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Abstract: Pathological aggression has a tremendous impact on its victims and society as a whole, which makes treatment of utmost importance. The serotonergic system has shown to be a successful target, as 5-HT_{1A} receptor agonists have shown to decrease aggressive behavior. Although only a subset of patients respond to these treatments, the importance of the serotonergic system in aggressive behavior is prominent. Differences in anti-aggressive pharmacological effects may be linked to behavioral traits, such as neuroticism and impulsivity. The mechanism of action of serotonergic drugs may be either by suppressing excessive bottom-up drives from the limbic regions or by restoring inadequate top-down control from prefrontal cortical areas (or both). In the present study we addressed whether such behavioral and biological characteristics are correlated with levels of aggressive behavior and 5-HT_{1A} receptor densities. We used an outbred strain of Long Evans rats in a resident-intruder test, elevated plus maze, autoshaping test and conditioned avoidance response task to evaluate aggression, anxiety and behavioral flexibility. Differences in 5-HT_{1A} receptor densities will be shown using imunnohistochemistry. We expect to make a distinction between different levels of aggression based on attack latencies and offensive behaviors in the resident intruder test. These differences in aggressive behavior will be used to classify the animals in groups of high and low aggression, so we might be able to correlate each class with a specific behavioral and biological make up. Preliminary data of this study will be shown on this poster.

Disclosures: **D. Peeters:** None. **H. Top:** None. **J. Homberg:** None. **R. Verkes:** None.

Poster

450. Defensive Behavior and Aggression

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 450.08/OO21

Topic: E.03. Behavioral Neuroendocrinology

Title: Immediate early gene activation in vasopressin 1b receptor knockout mice after an agonistic encounter

Authors: *S. K. WITCHEY[1], E. L. STEVENSON[1], H. K. CALDWELL[1,2]
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Abstract: The neuropeptide arginine vasopressin (Avp) is an important modulator of social behaviors, including social recognition memory and aggression via its two centrally expressed receptors, the Avp 1a receptor and the Avp 1b receptor (Avpr1b). Genetic disruption of the Avpr1b results in mild impairments in social recognition memory and reduced aggressive behavior. What has remained unknown is where in the brain these behavioral effects are being mediated. Interestingly, the Avpr1b is discretely localized within the central nervous system, being prominently expressed within the pyramidal cells of the CA2 region of the hippocampus. While recent work suggests that disruption of CA2 signaling results in impaired social recognition memory, whether this is Avpr1b-specific or if it is also important to aggressive behavior remains unknown. However, hippocampal lesion studies that have included the CA2 region report deficits in aggressive behavior similar to those observed in Avpr1b knockout (−/−) mice. Thus, we set out to examine differences in immediate early gene activation following an agonistic encounter in Avpr1b −/− and +/+ mice. Using either a resident-intruder test (males) or a maternal aggression test (females) as the stimulus, we looked at early growth response factor 1 (EGR-1) expression, using immunocytochemistry, in numerous brain regions. In females, there were no genotypic differences in EGR-1 expression in any of the brain areas measured. In males, there was a significant genotypic difference in EGR-1 expression within the ventral bed nucleus stria terminalis. These data suggest that genetic disruption of the Avpr1b in males has a measurable effect on what brain areas are “turned on” during an agonistic encounter. Whether or not these differences are due specifically to Avpr1b in the CA2 region of the hippocampus remains unknown.

Disclosures: S.K. Witchey: None. **E.L. Stevenson:** None. **H.K. Caldwell:** None.

Poster

450. Defensive Behavior and Aggression

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Program#/Poster#: 450.09/OO22

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS 1256898

Title: David vs. Goliath: Heightened serotonin increases aggressive behavior in smaller competitors and influences the larger competitor's fighting strategy

Authors: *A. BUBAK[1], N. S. RIEGER[2], K. J. RENNER[3], J. G. SWALLOW[2]
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Abstract: In aggressive encounters, size discrepancy between competitors is often a primary determining factor of contest outcome, usually resulting in the smaller competitor conceding to its larger rival. Because winning contests can lead to significant fitness advantages, understanding the mechanisms that alter aggression is of great importance. The stalk-eyed fly, *Teleopsis dalmanii*, aggressively defends food resources and roosting sites daily, with a high probability of males winning a contests when faced with a smaller rival (> 5% difference in eye span). Serotonin (5-HT) has been implicated in the escalation of aggressive behaviors in both invertebrates and vertebrates. Studies in our lab have demonstrated an increased probability of winning size-matched contests as well as increasing willingness to engage in high-intensity behaviors by pharmacologically elevating neural 5-HT in this species. We hypothesized that smaller flies with pharmacologically-increased brain 5-HT in a size-mismatched contest would show more aggressive behaviors and a greater win percentage compared to non-treated counterparts. To test this, size-mismatched males were placed in a 10-minute forced fight paradigm where the smaller fly was either treated or untreated with the 5-HT precursor, 5-hydroxytryptophan, and aggressive behaviors were scored. Although probability of winning was not significantly altered by the treatment, aggressive behaviors including contest initiation, total interactions, and high-intensity behaviors were significantly higher in treated animals. Interestingly, untreated larger opponents also demonstrated altered aggression by significantly increasing the initiations of high intensity behaviors in response to opponents with increased brain 5-HT, warranting future studies investigating the role of 5-HT in rival assessment in stalk-eyed flies.

Disclosures: **A. Bubak:** None. **N.S. Rieger:** None. **J.G. Swallow:** None. **K.J. Renner:** None.

Poster

450. Defensive Behavior and Aggression

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Title: 5-HT2A modulates the interaction between low MAO A activity and early childhood maltreatment on aggression pathogenesis

Authors: *S. C. GODAR[1], L. J. MOSHER[1], A. M. RUBY[1], S. SCHEGGI[2], C. GAMBARANA[2], M. DE MONTIS[2], M. BORTOLATO[1]
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Abstract: Ample evidence has shown that monoamine oxidase (MAO) A, the primary enzyme responsible for serotonin (5-HT) and norepinephrine degradation, plays a key role in pathological aggression. MAO A-deficiency in humans and rodents is associated with high levels of brain 5-HT and a greater severity of aggressive and antisocial traits, however, complete gene deficiency is very rare and may not present the optimal conditions to model aggression. In clinical studies, individuals carrying low MAO A activity polymorphic variants who were subjected to early maltreatment have a significantly higher risk for developing aggression and antisocial behavior. These findings highlight a complex interaction of gene (low levels of monoamine oxidase A) x environment (early neglect or abuse) in the pathogenesis of aggression. We recently generated a novel model based on this gene x environment interaction by subjecting non-aggressive MAO A hypomorphic mutant mice (MAO ANeo) to daily maternal separation (MS) from postnatal day 1 through postnatal day 7. Early MS markedly increased aggression in MAO ANeo, but not in WT mice. To investigate the neurobiological underpinnings of this interaction, we examined the role of the serotonin 5-HT2A receptor. We found that unstressed MAO ANeo mice exhibit low levels of 5-HT2A at postnatal day 7 compared to WT littermates.

In contrast, maternal separation significantly enhanced 5-HT2A receptor expression and binding. Moreover, selective 5-HT2A blockade during the first postnatal week significantly reduced aggression in MS-MAO ANeo mice. Taken together, these data provide a potential molecular mechanism to explain how low MAO A activity and early childhood maltreatment interact in the pathogenesis of aggression.

Disclosures: **S.C. Godar:** None. **L.J. Mosher:** None. **A.M. Ruby:** None. **S. Scheggi:** None. **C. Gambarana:** None. **M. De Montis:** None. **M. Bortolato:** None.

Poster

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Support: NSF IOS 0921874

Title: Inhibition of organic cation transporter 3 (OCT3) in the central nucleus of the amygdala increases extracellular serotonin and reduces fear expression

Authors: *J. E. HASSELL, JR[1,2], H. LI[1], J. ROGERS[1], S. FERRELL[1], M. ORCHINIK[3], C. A. LOWRY[2], K. J. RENNER[1]
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Abstract: Organic cation transporter 3 (OCT3) is a corticosterone-sensitive low affinity high capacity transport protein, expressed in neurons and glial cells, that clears monoamines, including serotonin (5-hydroxytryptamine; 5-HT) from the extracellular space. The central nucleus of the amygdala (CE) has major reciprocal connections from monoaminergic nuclei such as the dorsal raphe nucleus and has been implicated in fear-related behaviors. Organic cation transporter 3 has been found to be expressed in the CE but little is known about its role in fear-related behaviors. We hypothesized that OCT3 modulates fear-related behavior by controlling extracellular 5-HT concentrations in the CE. We predicted that inhibition of OCT3, under basal or restraint stress conditions, would elevate extracellular 5-HT concentrations and inhibit fear as part of a negative feedback loop. We tested this hypothesis with two experiments. In Experiment 1 rats received unilateral reverse dialysis of one of two different OCT3 blockers, either corticosterone (CORT) or normetanephrine (NM), into the CE, superimposed with a 40 min

period of restraint stress. We then analyzed extracellular 5-HT concentrations using high performance liquid chromatography with electrochemical detection. In Experiment 2 rats received bilateral microinfusions of vehicle, CORT or NM into the CE immediately before exposure to either home cage control conditions or restraint stress for 40 min. Subsequently rats were tested in the elevated plus-maze. Rats that received reverse dialysis of either CORT or NM superimposed with restraint stress, relative to reverse dialysis of vehicle, had significantly elevated levels of extracellular 5-HT measured using microdialysis. In addition, among rats exposed to home cage control conditions, but not among rats exposed to restraint, bilateral microinfusion of either CORT or NM into the CE decreased the duration of freezing behavior in the elevated plus-maze, without affecting the percent time spent exploring the open arms. These findings suggest a significant role for OCT3 in the CE in control of serotonergic signaling and fear-related behaviors.

Disclosures: **J.E. Hassell:** None. **H. Li:** None. **J. Rogers:** None. **S. Ferrell:** None. **M. Orchinik:** None. **C.A. Lowry:** None. **K.J. Renner:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIDA RO1-DA029613

Title: Anabolic-androgenic steroids impair decision making on a rodent version of the Iowa Gambling Task

Authors: ***K. G. WALLIN**[1,2], R. I. WOOD[1]
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Abstract: High-dose anabolic-androgenic steroids (AAS) induce aggression and dependence, but potential effects on cognition are understudied. In this regard, high testosterone levels correlate with financial risk taking, and AAS have been implicated in dopamine dysfunction in prefrontal cortico-striatal circuitry. The present study determined if AAS impair economic decision making. Male Long-Evans rats were treated chronically with testosterone (7.5 mg/kg) or vehicle, and tested for decision-making ability on a rodent version of the Iowa Gambling Task

(IGT). This operant task required rats to choose between four levers, analogous to the four decks of cards in the human IGT. The levers were associated with different reward probability, reward magnitude on wins, and time-out duration on losses. Two levers were advantageous (205 pellets/30-minute session); two levers were disadvantageous (122 or 125 pellets). Rats were tested for 22 days, and we compared selection of each lever by testosterone- and vehicle-treated rats over the last 3 days. By RM-ANOVA, there was a significant interaction of drug (testosterone vs vehicle) with lever selection ($F_{3,16}$=4.67, $p<0.05$). Testosterone-treated rats chose the two advantageous levers significantly less (14.2±3.5% of trials) than vehicle controls (62.5±10.2% of trials, $p<0.05$). As a result, testosterone-treated rats earned fewer pellets than vehicle controls (vehicle: 152.8±9.3 pellets, testosterone: 122.2±1.1, $p<0.05$). Testosterone-treated rats preferred the disadvantageous lever with a large reward magnitude (4 pellets/win) but a high cost of uncertainty and time-outs (percent of responses: vehicle 29.3±10.1%, testosterone 70.5±11.6%; $p<0.05$). During time-outs, testosterone-treated rats made significantly more responses on the inactivated lever (vehicle 0.05±0.01 responses/second, testosterone 0.18±0.02, $p<0.05$). This study suggests that testosterone impairs economic decision making by shifting preference to large magnitude rewards (pellets/win) despite high costs, resulting in fewer pellets earned over time. Supported by NIDA RO1-DA029613 to RIW.

Disclosures: K.G. Wallin: None. **R.I. Wood:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Title: Sex differences in the strength of projections from the orbital frontal cortex to the dorsal striatum in adult rats: Implications for sex differences in inhibitory control

Authors: *D. W. BAYLESS[1], J. M. DANIEL[1,2]
[1]Dept. of Psychology, [2]Program in Neurosci., Tulane Univ., New Orleans, LA

Abstract: Impulsive actions and decisions often lead to undesirable outcomes. Lesion and neuroimaging studies have revealed that the orbital frontal cortex (OFC) and dorsal striatum

(dSTR) play a key role in inhibitory control. It has been proposed that greater OFC control over the dSTR reflects enhanced top-down cognitive control and less impulsive responding. We previously reported a sex difference in inhibitory control, such that adult male rats make more impulsive errors than do female rats during the 5-choice serial reaction time task. In addition, we have demonstrated that adult female rats have increased levels of markers of myelination in the OFC but not the dSTR as compared to male rats. The goal of the present experiment was to determine if a sex difference in the strength of projections from the OFC to dSTR exists by infusing an anterograde tracer into the OFC and measuring the levels of tracer in the dSTR. The anterograde tracer biotinylated dextrane amine (BDA) was infused into the OFC of adult male and female rats. Brains were removed 10 days after infusion and coronal sections of the dSTR were collected. BDA expression was visualized using immmunohistochemistry followed by light microscopy imagining and densitometry analysis. Results revealed that the expression of BDA in the dSTR was significantly greater in adult female rats as compared to male rats indicating that the projections from the OFC to dSTR are greater in females as compared to males. This novel discovery provides a neuroanatomical sex difference that may contribute to the reported differences in inhibitory control levels of male and female rats.

Disclosures: D.W. Bayless: None. **J.M. Daniel:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Topic: E.03. Behavioral Neuroendocrinology

Title: Estrogen-dopamine interactions during extinction learning in female rats

Authors: *M. R. FARRELL, K. FLICK, J. M. LIPPS, R. M. SHANSKY
Dept. of Psychology, Northeastern Univ., Boston, MA

Abstract: Women are twice as likely as men to develop Post Traumatic Stress Disorder (PTSD), but the neurobiological factors underlying this discrepancy are mostly unknown. In preclinical studies using fear conditioning and extinction paradigms, female rats with low estrogen levels exhibit impaired extinction retrieval. We have shown previously that estrogen can modulate dopaminergic transmission to rescue extinction retrieval impairments, suggesting that estrogen-dopamine interactions may be important during fear extinction learning. However, the physiological effects of estrogen on dopamine (DA) transmission during fear extinction are

unknown. Intact female Long-Evans rats underwent a 2-day fear conditioning and extinction learning paradigm. Blood was drawn on the day of extinction learning for subsequent estradiol analysis, and estradiol levels were correlated with fear behavior during extinction. Immunohistochemistry for c-Fos and/or tyrosine hydroxylase (TH) was performed to compare neuronal activation during extinction learning in low- and high-estradiol rats. To uncover how estrogen modulated DA release in brain regions known to mediate extinction, we used immunohistochemistry for c-Fos and TH to quantify activation of DA neurons in the ventral tegmental area. To uncover physiological effects of estrogen on neuronal activity during extinction learning, c-Fos immunopositive cells were counted in medial prefrontal cortex and basolateral amygdala in low- and high-estradiol rats. This study demonstrated that the interaction of estrogen and DA modulated extinction learning by activating DA transmission in selective brain regions.

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Poster

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Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR MOP 102568

Title: Enzymatic cleavage of PSA-NCAM alters the effects of stress and fluoxetine treatment on neural activation within the dentate gyrus

Authors: *S. R. WAINWRIGHT[1], C. K. BARHA[2], D. K. HAMSON[2], U. RUTISHAUSER[4], L. A. M. GALEA[2,3]
[1]Grad. Program in Neurosci., [2]Psychology, [3]Brain Res. Ctr., Univ. of British Columbia, Vancouver, BC, Canada; [4]Mem. Sloan-Kettering Cancer Ctr., New York, NY

Abstract: Recent research has implicated adult hippocampal neurogenesis in the regulation of the hypothalamic-pituitary-adrenal axis in response to stress. Similarly, neurogenesis has been suggested to play a role in the re-establishment of basal corticosterone levels, and normal diurnal rhythm with chronic antidepressant treatment. However, neuroplasticity, beyond neurogenesis, including the enhancement of synaptic plasticity in the hippocampus, may also play a significant role in buffering the stress response. In accordance with a neuroplasticity theory of depression,

we sought to examine the role of PSA-NCAM in the re-establishment of basal CORT tone via chronic fluoxetine treatment, and in the integration and activation of newly generated neurons within the dentate gyrus (DG). Eighty adult male Sprague-Dawley rats received unilateral ICV infusions, with half receiving endoneuraminidase N (EndoN) (1µl/rat; 180U/µl) and the other half infused with saline. These groups were further sub-divided into Stress or Non-stress conditions, where the Stress group received six weeks of chronic variable stress (CVS) to induce a depressive-like behavioural phenotype. Each treatment group (n=10) received an injection of fluoxetine (Flx; 10mg/kg, i.p.) or saline daily for the final three weeks of the experiment. Sucrose preference was measured weekly, while at the completion of CVS application all rats were tested in the novelty suppressed feeding task, followed by the forced swim test (FST) to assess the antidepressant efficacy of Flx. Ninety minutes after the completion of FST rats were sacrificed and brain tissue was collected and processed. As expected EndoN treatment greatly reduced the number of PSA-NCAM labelled cells. EndoN treatment was also seen to decrease BrdU labelled cells in the GCL indicating decreased cell survival, and reduced the volume of the granule cell layer (GCL). Interestingly, EndoN treatment also altered cFos expression in the GCL, where Flx treatment failed to reduce cFos expression in stressed rats receiving EndoN. Further cytological and endocrine measures will be explored to assess the effects of Flx treatment and PSA-NCAM cleavage on the stress response and cell activation.

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Poster

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Support: CIHR MOP 102568

Title: Estradiol and activation of the membrane receptor GPER decrease GPER expression but have opposing effects on cell proliferation in the hippocampus of adult female rats

Authors: *C. CHOW[1], P. DUARTE-GUTERMAN[2], S. E. LIEBLICH[2], L. A. M. GALEA[2]
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Abstract: Estradiol exerts its effects via both nuclear and membrane-bound estrogen receptors (ERs). In adult female rats, estradiol rapidly upregulates cell proliferation in the dentate gyrus of the hippocampus. Interestingly, these effects cannot be completely explained by the actions of the nuclear ERs, ERα and ERβ, alone, as ERα and ERβ agonists did not increase cell proliferation to the same extent as estradiol alone, and the nuclear ER antagonist ICI 182, 780 did not completely block estradiol-induced enhancement of cell proliferation. Therefore, nuclear ERs either operate in concert with or independently of other mechanisms to influence cell proliferation. One such mechanism may be the G protein-coupled estrogen receptors (GPERs), which are membrane receptors found throughout the hippocampus, including the dentate gyrus. The location of these receptors coupled with the rapid upregulation of cell proliferation by estradiol implicates a role for this receptor in regulating adult hippocampal neurogenesis. In the current study, we examined whether the rapid effects of estradiol on cell proliferation are exerted through GPERs. Ovariectomized adult female rats received a single injection of either: 17β-estradiol (10μg; s.c), GPER agonist G1 (0.1, 5, or 10μg; i.p), GPER antagonist G15 (40μg; i.p), G15+estradiol, or vehicles (oil, DMSO, or oil+DMSO). Thirty minutes later, animals were injected with the DNA synthesis marker, bromodeoxyuridine (BrdU; 200mg/kg; i.p), and sacrificed 24hrs later. As expected, estradiol increased the number of BrdU positive cells in the dorsal dentate gyrus relative to controls. Interestingly, the low and medium doses of G1 significantly decreased cell proliferation relative to controls, while G15 alone increased cell proliferation and when co-administered with estradiol, failed to block the estradiol-induced increase in cell proliferation. Using optical density, we examined the distribution of GPER in the dentate gyrus. The estradiol and G1 (medium and high dose) groups had significantly lower expression of GPER in the dorsal region relative to vehicle controls. In contrast, G15 alone significantly increased the expression of GPERs relative to the vehicle and estradiol groups in the dorsal dentate gyrus. Altogether, these results suggest that estradiol's rapid effects on hippocampal neurogenesis are not exerted through GPERs, but through other pathways. However, estradiol and G1 seem to regulate dentate gyrus GPER expression in a similar way. Finally, the effects of GPER activity on cell proliferation uncovers a new mechanism via which adult neurogenesis is regulated in adult female rats. Funded by CIHR (MOP 102568)

Disclosures: C. Chow: None. **P. Duarte-Guterman:** None. **S.E. Lieblich:** None. **L.A.M. Galea:** None.

Poster

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Title: Sex differences in adult neurogenesis and IEG expression in the hippocampus after a spatial pattern separation task

Authors: *S. YAGI[1,2], S. E. LIEBLICH[3,4], L. A. M. GALEA[2,4,3]
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Abstract: Adult neurogenesis in the dentate gyrus (DG) plays a critical role for pattern separation, the process of forming distinct representations of similar inputs during memory encoding. The objectives of the study were to determine whether there are sex differences in the ability for separating similar patterns, learning strategy choice and to determine IEG expression and adult neurogenesis in the DG in response to pattern separation training. Male and female Sprague-Dawley rats received a single bromodeoxyuridine (BrdU) injection on day 1 and were tested in the spatial pattern separation paradigm for 15 days beginning on day 14 after BrdU. Rats were tested in a delayed nonmatching to place with radial 8-arm maze. During the sample phase, all arms except the start arm and the sample arm were blocked off and a rat was allowed to enter the sample arm and retrieve a food pellet reward. Forty seconds after the sample phase, an additional arm opened and the rat was allowed to choose one arm from the sample arm or the new correct arm. Two patterns of correct arms were different in angle from the sample arm. One separation pattern was 45 degrees away (separation 1) and the other pattern was 135 degrees away (separation 2) from the sample arm. Rats were perfused 90 minutes after the last trial on day 28. Ten minutes before perfusion, rats were examined whether they were idiothetic cue users or external cue users with a probe trial. We found that female rats chose less correct arms than male rats in separation 1. Sex had no significant effect on learning strategy choice, although there was a trend for proestrous rats to be external spatial cue users. Female external spatial cue users chose less correct arms in separation1 than in separation2. Conversely, male rats showed no significant differences in their performance between separation 1 and separation 2. Male rats had a significant greater density of BrdU-labelled cells than female rats and male idiothetic cue users showed greater BrdU-labelling than female idiothetic cue users. Furthermore, male idiothetic cue users, but not external spatial cue users or females, had significant negative correlations between ventral density of BrdU-labelled cells and accuracy in separation1. In conclusion, male rats performed better at separating similar spatial stimuli than female but not at distinct spatial stimuli. The correlation of adult neurogenesis to the performance is limited to specific strategy users and sex. Further analysis with zif268 immunohistochemistry is ongoing to examine the correlation of activation of neurons in the DG to the performance.

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Poster

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NIH PO1 AG024387-04

Title: The ERα agonist PPT enhances place learning but impairs response learning in ovariectomized young adult rats: Viewing the role of ERK activation through a multiple memory systems lens

Authors: *S. L. PISANI[1], D. L. KOROL[1,2]
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Abstract: Estrogens have opposing effects on cognition, with increased hormone levels generally enhancing hippocampus-sensitive functions such as place learning and impairing striatum-sensitive processes such as response learning. We have shown that estrogens exert these mnemonic actions via local estrogen receptors (ERs) in each canonical structure. Moreover, estrogens modulate patterns of ERK signaling in a structure- and performance-specific manner: activation of ERK in the hippocampus appears dominant during early phases of learning, but activation in the striatum increases at later phases of task acquisition, possibly reflecting a shift to habit-based strategies. Reports by others link ERα activation to improvements in hippocampus-sensitive memory and phosphorylation of extracellular signal-regulated kinase (ERK). Recently we found that systemic treatment with the ERα-selective agonist PPT mimics estradiol-induced shifts in learning strategies, as PPT improved place learning at an intermediate dose (333 μg/kg) only and slowed acquisition of the response task at the highest doses (333 and 1000 μg/kg). Here, we test whether local changes in ERK activation in the hippocampus and striatum align with the magnitude and direction of our observed learning shifts after ERα activation by PPT. Separate groups of young adult ovariectomized rats were treated with one of four doses of PPT (33, 100, 333, or 1000 μg/kg) or vehicle and trained on either place or response learning tasks. The hippocampi and striata were harvested immediately after training

for 100 trials and processed for biochemical assays. ERK activation was measured in each neural structure using quantitative Western blot analyses. For the place task, ERK activation largely did not correlate with learning performance or PPT treatment. Interestingly, a decrease in hippocampus ERK phosphorylation was observed at the highest dose of 1000 μg/kg PPT. This corresponded to an inverted-U pattern of place learning enhancement where the improved performance at 333 μg/kg PPT was lost at 1000 μg/kg. Our findings align with other *in vitro* data of attenuated MAPK signaling in hippocampal neurons following treatment with ERα agonists during K+-induced activation, a condition that may simulate hippocampal activation states during place learning. We are currently investigating phosphorylation of ERK in the hippocampus and striatum following response training and PPT treatment. Together, these data will advance understanding of the cellular mechanisms by which estrogens shift cognitive strategies according to actions in discrete memory systems.

Disclosures: S.L. Pisani: None. **D.L. Korol:** None.

Poster

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Support: NIH Grant R01AG041374

Title: Prior estradiol exposure in midlife protects hippocampal estrogen receptor alpha from C terminus of Hsc70-interacting protein (CHIP)-mediated degradation in aging, ovariectomized rats

Authors: *K. L. BLACK[1], R. C. SPRINGER[1], J. M. DANIEL[1,2]
[1]Neurosci. Program, [2]Psychology Dept., Tulane Univ., New Orleans, LA

Abstract: The long-term implications of short-term estrogen use in midlife on cognition and the aging female brain are unknown. Work from our lab demonstrates that in aging, ovariectomized rats, previous exposure to 40 days of chronic estradiol administration in middle-age results in lasting memory enhancements. Furthermore, midlife estradiol exposure results in lasting increases in levels of estrogen receptor alpha (ERα) in the hippocampus, an effect which mediates the memory enhancements. The goal of our current work is to identify mechanisms by which increased levels of ERα resulting from midlife estradiol administration are maintained

beyond the period of estradiol exposure. There is evidence that in the absence of ligand, ERα is bound by the ubiquitin ligase, C terminus of Hsc70-interacting protein (CHIP), which promotes its degradation by the ubiquitin-proteasome pathway. The current experiment tests the hypothesis that previous estradiol exposure leads to decreased CHIP-mediated ERα degradation, as indicated by decreased association between ERα and CHIP. Middle-aged rats were ovariectomized and received a subcutaneous implant of either estradiol or cholesterol vehicle. After 40 days, all implants were removed. Twenty-five days after implant removal, rats were killed and hippocampi processed for co-immunoprecipitation and western blot analysis during which samples were immunoprecipitated with anti-ERα antibody and subsequently probed for CHIP. Additionally, whole cell homogenate taken from the same hippocampi was used to measure total levels of ERα and CHIP. Co-immunoprecipitation results revealed decreased association between CHIP and ERα in ovariectomized rats previously exposed to estradiol as compared to controls. Furthermore, consistent with our prior results, rats previously exposed to estradiol had increased total levels of ERα in the hippocampus as compared to controls. No differences in total levels of CHIP were found. These data indicate that prior exposure to estradiol protects ERα in the hippocampus from CHIP-mediated degradation, leading to maintained increased levels of ERα in the aging female.

Disclosures: **K.L. Black:** None. **R.C. Springer:** None. **J.M. Daniel:** None.

Poster

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NIH P30 AG034464

Title: Bioenergetics and Memory: Regulation by Estradiol

Authors: ***W. WANG**, B. T. YUHAN, D. L. KOROL, P. E. GOLD
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Abstract: Estradiol, the predominant estrogen in females, shifts the strategies used by rats during learning and memory: Rats with high estrogen levels excel on hippocampus-based tasks but are impaired on striatum-based tasks, and rats with low estrogen levels excel on striatum-based tasks but do poorly on hippocampus-sensitive tasks (Korol & Kolo, Behav. Neurosci., 2002). Furthermore, it is thought that depletion of circulating estrogens after ovariectomy impairs glucose import into the brain but may enhance delivery of lactate from astrocytes (Ding et al., PlosOne, 2013). Lactate converted from glycogen and shuttled from astrocytes can serve the energy needs of neurons particularly when energy demands are high and glucose supply is insufficient. These converging lines of evidence prompted us to hypothesize that the bioenergetic demands of learning and memory in females are mediated by estrogens. To compensate for the loss of glucose uptake, ovariectomized rats without hormone exposure may have higher baseline levels of extracellular lactate in the hippocampus than do rats with estradiol treatments. Because estrogens promote hippocampus-based learning, we hypothesize that estrogen-deprived rats would also show reduced extracellular lactate responses to training. In the present study, ovariectomized 3-month-old rats received either estradiol (45 μg/kg) or vehicle oil (1 mL/kg) injected 24 and 48 hours prior to testing. Lactate oxidase-coated biosensor probes were implanted unilaterally in the dorsal hippocampus to measure extracellular lactate concentrations before and during 20-min tests of spatial working memory on a spontaneous alternation task. While both groups alternated significantly above chance, scores did not differ by estradiol treatment, perhaps because the spontaneous alternation task engages multiple memory systems during testing. Strikingly, both groups exhibited significant increases in hippocampal extracellular lactate concentrations during memory testing, indicating that the memory tests initiated release of lactate from astrocytes in female rats as seen before in male rats. However, peak change in lactate concentrations during testing did not differ significantly by treatment, perhaps reflecting the similar memory scores in the two treatment groups. We next plan to test the effects of estrogens on learning strategies and brain bioenergetics in tasks that are more specifically associated with selective processing in the hippocampus, striatum, and other brain regions. Results from these experiments will lend insight into how estrogens shift brain metabolism across multiple memory systems to modulate cognition.

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Program#/Poster#: 451.10/PP2

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC grant 301 029400 400212 000000

Title: The rapid effects of the combined activation of both the G-protein coupled estrogen receptor and estrogen receptor alpha in the hippocampus on learning and memory in female mice

Authors: *J. LYMER[1], A. PHAN[2], A. ROBINSON[1], P. ALEX[1], E. CHOLERIS[1]
[1]Psychology, Univ. of Guelph, Guelph, ON, Canada; [2]The Scripps Res. Inst., Jupiter, FL

Abstract: There is increasing evidence that estrogens, such as 17β-estradiol are involved in rapidly mediating different learning and memory paradigms through at least three estrogen receptors: estrogen receptor (ER) α, ERβ (Phan et al., 2011, 2012), and the recently discovered G-protein coupled estrogen receptor (GPER). Both systemic and intrahippocampal administration of 17β-estradiol, the ERα agonist, PPT, or the GPER agonist, G-1, improves social recognition, object recognition, and object placement learning while administration of the ERβ agonist, DPN, improves only object placement learning in ovariectomized female mice. These paradigms were completed within 40 minutes of drug administration, thus demonstrating the rapid effects of estrogens, specifically through receptors in the hippocampus on learning and memory. Whether these receptors are working through separate or additive mechanisms is unknown. This study investigates the combined action of hippocampal GPER and ERα on social recognition, object recognition, and object placement learning. Sub-effective doses of both G-1 and PPT were simultaneously infused directly into the CA1 of the hippocampus of ovariectomized female mice 15min before testing in one of the three learning and memory paradigms. The paradigms consist of two 5min habitation phases, where the mice are presented with two stimulus mice or objects and one 5 min test phase, where one of the two stimuli is replaced with a novel one, with 5 min intertest intervals. In the test phase of the object placement paradigm, one of the two familiar objects is moved to a novel location. The object recognition experiment is currently underway. Social recognition was improved with the combined infusion of the sub-effective doses of G-1 (100nM) and PPT (50nM), while infusion of those doses of G-1 and PPT alone did not improve social recognition. However, the combined infusion of the sub-effective doses of G-1 (50nM) and PPT (50nM) did not improve performance in the object placement task. Therefore, estrogens appear to be mediating social recognition but not object placement learning through the additive mechanisms of both the GPER and ERα in the hippocampus. Supported by NSERC.

Disclosures: J. Lymer: None. **A. Phan:** None. **A. Robinson:** None. **P. Alex:** None. **E. Choleris:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.11/PP3

Topic: E.03. Behavioral Neuroendocrinology

Support: The University of Wisconsin-Milwaukee College of Letters and Sciences (KMF)

WM Research Foundation Research Growth Initiative grant (KMF)

Title: Distinct effects of estrogen receptor inhibition on novel object recognition and spatial memory consolidation in ovariectomized mice

Authors: *J. KIM, J. S. SZINTE, K. M. FRICK
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The memory-enhancing effects of 17β-estradiol (E2) in ovariectomized female rodents are likely mediated by a combination of intracellular (ERα and ERβ) and/or membrane-bound estrogen receptors (e.g., GPER, G-protein coupled estrogen receptor). Administration of E2 or selective estrogen receptor (ER) agonists enhances hippocampal memory consolidation in novel object recognition (NOR) and object placement (OP) tasks. However, the extent to which individual ERs are necessary for memory consolidation in these tasks is not well un-derstood. Therefore, the present study examined the effects on NOR and OP memory con-solidation of dorsal hippocampal infusion of selective antagonists for ERα, ERβ, and GPER. Ten week-old ovariectomized C57BL/6 female mice received bilateral dorsal hippocampal infusion of vehicle, the selective ERα antagonist MPP (1,3-Bis(4-hydroxyphenyl)-4methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole), the selective ERβ antagonist PHTPP (4-[2-Phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol), or the selective GPER antagonist G-15 immediately after NOR or OP training. Memory was tested 24 hours later for NOR and 4 hours later for OP. Each drug exhibited a distinct effect on NOR and OP, alt-hough both tasks involve the dorsal hippocampus. Lower doses of MPP impaired OP memory but not NOR memory, although a high dose of MPP impaired both OP and NOR memory. A high dose of G-15 impaired both OP and NOR memory, and a middle dose im-paired NOR memory only. All doses of PHTPP tested impaired NOR memory; studies of OP memory are currently ongoing. These data suggest that intracellular ERs and GPER are necessary for some forms of hippocampal memory consolidation, but that their involvement depends on the type of memory tested. Collectively, the present study provides new insights into the ER mechanisms modulating hippocampal memory consolidation in females.

Disclosures: **J. Kim:** None. **J.S. Szinte:** None. **K.M. Frick:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.12/PP4

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR (MOP 102568)

Title: Gonadal hormone status predicts depressive-like behaviour in middle-aged female rats

Authors: ***R. MAHMOUD**, S. R. WAINWRIGHT, S. E. LIEBLICH, L. A. M. GALEA
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Women are twice more likely than men to suffer from depression, and sex differences are evident in antidepressant efficacy. These findings suggest a role of gonadal hormones in the development of depression and in antidepressant efficacy. Despite this, male animals are almost exclusively used in preclinical depression research, hindering our full understanding of the disease pathoetiology and the mechanisms underlying antidepressant efficacy. The purpose of this study was to test the effect of hypogonadism on the development of depressive-like phenotypes and on antidepressant efficacy in middle-aged female Spague-Dawley rats. We exposed ovariectomized (OVX) and sham-operated rats to 6-weeks of chronic variable stress (CVS); a protocol commonly used to induce depressive-like phenotypes in rodent. At the start of week 4, rats received chronic daily injections of a sub-threshold dose of fluoxetine (FLX) or vehicle. All rats were assessed on tasks that measure depression- and anxiety-like behaviour. OVX rats displayed more passive and less active behaviour than sham-operated rats in the forced swim test, and had a higher latency to feed in the novelty suppressed feeding task. However, regardless of ovarian hormone status, CVS exposure did not produce anhedonia-like phenotype as measured in the sucrose preference test. Interestingly, regardless of ovarian hormone status, fluoxetine treatment did not attenuate anxiety- or depression-like behaviour. Furthermore, we administered the dexamethasone suppression test to assess hypothalamic-pituitary-adrenal axis function, and we analyzed changes in hippocampal neuroplasticity (cell proliferation and neurogenesis). Our findings show that ovarian hormones protect females from the deleterious effects of chronic variable stress, but do not enhance efficacy of sub-threshold fluoxetine treatment.

Disclosures: **R. Mahmoud:** None. **S.R. Wainwright:** None. **S.E. Lieblich:** None. **L.A.M. Galea:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Topic: E.03. Behavioral Neuroendocrinology

Support: University of Wisconsin-Milwaukee College of Letters and Sciences funding to KMF

PSC-CUNY 66720-44 grant to VL

Title: Dorsal hippocampal infusion of 17β-estradiol increases dendritic spine density in the CA1 subfield of the hippocampus in ovariectomized female mice

Authors: *J. J. TUSCHER[1], M. FRANKFURT[2], V. LUINE[3], K. M. FRICK[1]
[1]Psychology Dept., UW-Milwaukee, Milwaukee, WI; [2]Dept. of Sci. Educ., Hofstra North Shore-LIJ Sch. of Med., Hempstead, NY; [3]Dept. of Psychology, Hunter Col. of the City Univ. of New York, New York, NY

Abstract: Dendritic spines increase the surface area available for the synaptic connections thought to underlie the formation and maintenance of long-term memories. Hippocampal spine remodeling is critical for the initiation of long-term potentiation, and increases in hippocampal CA1 dendritic spine density are believed to contribute to memory. In ovariectomized (OVX) rats, systemic injections of 17β-estradiol (E2) increase the density of dendritic spines in the CA1 region of the hippocampus and in the medial prefrontal cortex (mPFC) 30 minutes and 4 hours after treatment. In OVX mice, chronic systemic E2 injection increases the number of mushroom-shaped spines in CA1. Also in mice, infusion of E2 bilaterally into the dorsal hippocampus enhances hippocampal-dependent novel object recognition and spatial memory consolidation by rapidly activating (within 5 minutes) ERK-dependent signaling of mammalian target of rapamycin (mTOR), a key protein synthesis pathway involved in spine remodeling. However, the extent to which intrahippocampal E2 infusion may regulate spine density in the hippocampus and it's projection regions, such as the mPFC, is not known. To address this issue, female C57BL/6 mice (8-10 weeks old) were OVX and implanted with bilateral guide cannulae in the dorsal hippocampus. One week after surgery, mice were infused bilaterally into the dorsal hippocampus with vehicle or a dose of E2 (5 μg/side) previously shown to enhance novel object

recognition and object placement memory consolidation in OVX mice. Brains were collected 30 minutes and 2 hours later for assessment of apical and basal spine density on pyramidal cells in CA1 and the mPFC by Golgi impregnation techniques. Infusion of E2 directly into the dorsal hippocampus significantly increased spine density on apical and basal dendrites in the CA1 30 minutes later by 15 and 30%, respectively. In contrast, E2 did not affect spine number in the dentate gyrus. E2 increased apical and basilar spine number in the mPFC by approximately 10% 30 minutes after infusion, but this increase did not reach statistical significance. Thus, significant spine changes appear confined to the CA1 area 30 min following infusion. The effects of E2 infusion on spines 2 hours after infusion are currently being measured. Thus far, the data show that intrahippocampal infusion of E2 is sufficient to rapidly increase dendritic spine density in CA1 and support previous observations that rapid increases in CA1 spine density may contribute to memory consolidation.

Disclosures: J.J. Tuscher: None. **M. Frankfurt:** None. **V. Luine:** None. **K.M. Frick:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.14/PP6

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant AG041374

Title: Effects of GnRH or GnRH plus the aromatase inhibitor letrozole on hippocampus-dependent memory and levels of hippocampal synaptic proteins in ovariectomized rats

Authors: *B. S. NELSON[1], J. M. DANIEL[1,2]
[1]Neurosci., [2]Psychology Dept., Tulane Univ., New Orleans, LA

Abstract: Increasing evidence indicates that GnRH plays a role in cognition. Additionally, data indicate both *in vivo* and *in vitro* that GnRH treatment to the hippocampus increases both pre and postsynaptic markers. *In vitro* evidence suggests that the effects of hippocampal GnRH treatment on these synaptic markers are dependent on its ability to stimulate neuroestradiol production in the hippocampus. Consequently, it is hypothesized that the GnRH-induced increase of neuroestradiol production ultimately affects cognition and hippocampal morphology. The goals of the current experiments were to test the hypothesis that hippocampal GnRH receptor activation affects hippocampus-dependent memory and synaptic markers in the hippocampus and to determine whether or not these effects are dependent on neuroestradiol production. In the initial experiment, thirty young adult female rats were ovariectomized and trained on a radial-

arm maze working memory task. After training, rats then underwent stereotaxic surgery whereby aCSF vehicle, GnRH (16.6 ng /hr) or GnRH (16.6 ng/hr) + letrozole, an aromatase inhibitor (31.5 ng/hr), was chronically delivered bilaterally in the dorsal hippocampus (0.25 µl/hr). After five days of recovery, rats were tested on the maze and sacrificed 10-12 days after initiation of drug treatment. Rats treated with GnRH outperformed rats that were treated with aCSF. Further, rats treated with GnRH + letrozole performed significantly worse than rats treated with GnRH alone. Unexpectedly, GnRH treatment significantly decreased levels of hippocampal PSD-95, a postsynaptic marker, as compared to aCSF treatment. Additionally, GnRH or GnRH + letrozole had no effects on hippocampal levels of spinophilin or synaptophysin. In a second experiment 14 rats were ovariectomized. After one week recovery, all rats underwent stereotaxic surgery whereby aCSF vehicle was infused at one side of the dorsal hippocampus and GnRH or GnRH + letrozole was infused in the remaining side at doses and rates identical to the first experiment. As a result, each rat represented its own control. After five days of treatment, which represents the time point at which rats began behavioral testing in the first experiment, rats were sacrificed and their dorsal hippocampi were processed for western blotting. GnRH treated sides had significantly higher levels of PSD-95 and spinophilin but not synaptophysin as compared to GnRH + letrozole treated sides. Results of these experiments support a role for hippocampal GnRH treatment on spatial memory and hippocampal morphology and suggest that these effects are dependent on the ability of GnRH to affect brain-derived estradiol synthesis.

Disclosures: B.S. Nelson: None. **J.M. Daniel:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.15/PP7

Topic: E.03. Behavioral Neuroendocrinology

Support: Foreign research program

Title: Neonatal exposure to ethinyl estradiol decreased the passive avoidance performance and the expression levels of ERα in the cortex and hippocampus adult female rats

Authors: *T. SHIGA[1], T. J. NAKAMURA[2], Y. MIZOGUCHI[1], C. KOMINE[3], Y. GOTO[4], M. KAMISHIMA[3], M. YOSHIDA[5], Y. KONDO[6], M. KAWAGUCHI[3]
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of Agriculture, Meiji Univ., 1-1-1 Higashi Mita Tamaku Kawasaki, Japan; [4]Fac. of Pharmaceut. Sciences, Teikyo Heisei Univ., 4-21-2 Nakano Nakano-ku, Tokyo, Japan; [5]Div. of Pathology, Natl. Inst. of Hlth. Sci., 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, Japan; [6]Sch. of Animal Sci., Fac. of Life and Envrn. Sciences, Teikyo Univ. of Sci., 2-2-1 Senjusakuragi Adachi-ku, Tokyo, Japan

Abstract: Although it is well known that perinatal exposure of estrogen to female rats produces irreversible changes in brain function, effects of estrogenic compounds, such as ethynyl estradiol (EE), exposure on learning function are still unclear. In this study, we investigated the effects of neonatal exposure to EE on passive avoidance and expression of estrogen receptor α (ERα) in the cortex (CTX) and hippocampus (HIP) in female rats. New born female rats of Wistar-Imamichi strain were subcutaneously administrated with vehicle (Oil), 0.02 mg/kg EE (LEE), 2 mg/kg EE (HEE), or 20 mg/kg 17β-estradiol (E2) within 24 hours after birth. In the first experiment, females of all groups were tested for passive avoidance learning at 6 weeks old. In the second experiment, other females of all groups were ovariectomized under pentobarbital anesthesia at 10 weeks old. At 15-17 weeks old, they were tested for passive avoidance learning, in which half of them received a subcutaneous injection of 5 μg estradiol benzoate (EB) on a day before the test. Neonatal LEE significantly disrupted passive avoidance than Oil treatment in gonadally intact pubertal females, while the deterioration in neonatal LEE was appeared only under EB treatment in sexually mature ovariectomized females ($P < 0.05$). We also assessed the expression of ERα protein in the CTX and HIP by western blotting in 17-19 weeks old females with or without 5 μg EB treatment. Brain tissues were collected from the females under lethal pentobarbital anesthesia one day after the treatment. The results showed a significant decrease in ERα expression of the HIP in the EB-injected rats by the neonatal EE treatment ($P < 0.05$). These suggest that exposure to EE immediately after birth decreases learning ability in female rats. We speculated that the disturbance of learning ability in EE-exposed females may be mediated by decreased expression of ERα in the HIP.

Disclosures: T. Shiga: None. **M. 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.; H25-KAGAKU-IPPANN-003, Research on Risk of Chemical Substances, Health and Labour Sciences Research Grants, Ministry of Health , Labour and Welfare, Japan. **C. Komine:** None. **M. Kamishima:** None. **M. Yoshida:** 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.; H25-KAGAKU-IPPANN-003, Research on Risk of Chemical Substances, Health and Labour Sciences Research Grants, Ministry of Health , Labour and Welfare, Japan. **T.J. Nakamura:** None. **Y. Goto:** None. **Y. Kondo:** None. **Y. Mizoguchi:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.16/PP8

Topic: E.03. Behavioral Neuroendocrinology

Support: Whitehall Grant

Title: Estradiol induces generalization of fear memories to neutral cues through estrogen receptor β

Authors: *J. F. LYNCH, III, P. A. WINIECKI, T. VANDERHOOF, S. ORTIZ, J. LONDON, D. C. RICCIO, A. M. JASNOW
Psychology, Kent State Univ., Kent, OH

Abstract: Females are 60% more likely to suffer from an anxiety disorder than males. Generalization is a common symptom of many anxiety disorders, thus, one hypothesis for the large sex difference in anxiety disorder rates may be that females exhibit higher rates of fear generalization than males. Indeed, our previous research demonstrated that female rats generalize learned fear to novel contextual cues at a faster rate than males and this effect is mediated by estradiol interactions with memory retrieval. The current set of experiments attempt to determine specific mechanisms by which estrogens affect memory retrieval to elicit generalized fear. First, we attempt to determine whether estradiol induces fear generalization through activation of traditional nuclear estrogen receptors using central infusions of the nuclear estrogen receptor antagonist, ICI 182,780. Female rats will be ovariectomized and given injections of estradiol 24 hours following passive avoidance training and given an infusion of ICI 182,780 into the lateral ventricle and tested 24 hours later in either the training context or a neutral context. In a second experiment, animals will be injected with the ERB agonist, DPN, to confirm the role of ERB activation in fear generalization. Previous research has implicated changes in the DG-CA3 circuit as being critical to fear generalization. We therefore attempt to determine estradiol-induced dendritic growth within the CA3 region using Golgi staining at times when generalization is observed with estradiol treatment. These experiments will help identify specific mechanisms through which estrogens are acting to enhance the generalization of fear.

Disclosures: J.F. Lynch: None. **P.A. Winiecki:** None. **T. Vanderhoof:** None. **S. Ortiz:** None. **J. London:** None. **D.C. Riccio:** None. **A.M. Jasnow:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant No. 0951008

NIH Grant No. R01AG041372

Title: Influence of estradiol on the ability of chronic IGF-I treatment to impact levels of hippocampal synaptic proteins and IGF-I receptors in ovariectomized rats

Authors: *M. R. VAN ROIJEN[1], B. S. NELSON[2], C. F. WITTY[2], M. N. MAINGUY[2], P. K. JHITA[2], K. M. LEE[2], J. M. DANIEL[1,2]
[1]Dept. of Psychology, [2]Neurosci. Program, Tulane Univ., New Orleans, LA

Abstract: The ability of insulin-like growth factor-I (IGF-I) to impact the hippocampus and associated behaviors may vary depending upon estrogenic status. Previous work from our lab demonstrated that chronic antagonism of brain IGF-I receptors (IGF-IR) resulted in increased levels of hippocampal synaptic proteins in control-treated ovariectomized (OVX) rats. In contrast, antagonism of brain IGF-IR decreased levels of synaptic proteins in estradiol-treated OVX rats. The goal of the current experiment was to test the hypothesis that effects of chronic agonism of brain IGF-IR via treatment with IGF-I on synaptic proteins would also vary with estrogenic status. Furthermore, we assessed the influence of estrogenic status on the ability of IGF-I to regulate its own receptor. In an initial experiment, OVX rats received chronic aCSF or IGF-I treatment delivered into the lateral ventricle. In a second experiment, estradiol-treated OVX rats were infused with aCSF or IGF-I. After six days, hippocampi were processed for western blotting. Chronic agonism of brain IGF-IR resulted in decreased levels of hippocampal PSD-95 in control-treated OVX rats, and increased levels of hippocampal PSD-95 in estradiol-treated OVX rats. Levels of hippocampal spinophilin and synaptophysin were not significantly influenced by IGF-I in either group. Hippocampal IGF-IR levels were decreased following chronic agonism of brain IGF-IR in control-treated OVX rats and increased in estradiol-treated OVX rats. Results indicate that chronic agonism of brain IGF-IR differentially impacts levels of hippocampal PSD-95 and IGF-IR in OVX rats depending upon estrogenic status. The effects of IGF-I may therefore be contingent on estradiol exposure, which suggests that crosstalk between IGF-I, estradiol, and their associated receptors has important implications in hippocampal-dependent learning and memory.

Disclosures: **M.R. Van Roijen:** None. **B.S. Nelson:** None. **C.F. Witty:** None. **M.N. Mainguy:** None. **P.K. Jhita:** None. **K.M. Lee:** None. **J.M. Daniel:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.18/PP10

Topic: E.03. Behavioral Neuroendocrinology

Title: Levonorgestrel and ethinyl estradiol alter novel object recognition and spatial memory in female rats

Authors: ***J. SIMONE**, D. BHATTI, P. V. HOLMES
Neurosci., Univ. of Georgia, Athens, GA

Abstract: Estimates of contraceptive hormone use among women in the U.S. exceed ten million. Levonorgestrel (LNG) and Ethinyl Estradiol (EE) have been mainstay contraceptive hormones for the last four decades. Surprisingly, there is little information regarding their effects on the central nervous system and behavior. Two doses of ethinyl estradiol (10 or 30 μg/rat/day) and levonorgestrel (20 or 60 μg/rat/day) were administered subcutaneously to intact female rats for three weeks, and rats were subsequently tested in three models of learning and memory, novel object (NOR), place (NOP) and context (NOC) recognition. Additionally, they were tested for exploratory activity in the open field (OF) and ataxia on the rota-rod (RR). To assess suppression of the hypothalamic-pituitary-gonadal axis, serum estradiol and ovarian weights were measured. The low estradiol levels and diminished ovarian weights in the vehicle group suggested that these rats were in the met-diestrus phase. All drug treatments similarly lowered estradiol levels. No significant difference was seen between groups in percent time spent in the perimeter or center of the OF suggesting that exploratory behavior was not affected by the drug treatments. In the RR, latency to fall was significantly increased in the higher dose EE group. Significant effects of drug treatment were observed in the NOR and NOC tests but not in the NOP. Low dose EE impaired NOR, whereas, the higher dose EE improved performance. Low dose LNG improved, while the higher dose impaired performance in NOC. The results suggest that the higher dose of EE (equivalent to 25 μg human dose), having potent estrogen receptor α (ERα) affinity, may be able to compensate for the low serum estradiol levels and thus improve learning and memory in NOR as seen in proestrus rats. Whereas LNG, having increased progesterone receptor affinity as well as selective ERα affinity, improves spatial memory at low doses

(equivalent to 16µg human dose) only. Future studies of neuropeptides, neurotrophins and synaptic architecture in these rats are underway to evaluate neurobiological mechanisms for the observed behavioral effects.

Disclosures: J. Simone: None. **D. Bhatti:** None. **P.V. Holmes:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Program#/Poster#: 451.19/PP11

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC

FRSQ

CFI

Title: Low chronic estradiol facilitates haloperidol to restore deficits in reversal learning

Authors: *A. ALMEY, J. OLIEL, L. ARENA, W. G. BRAKE
Concordia Univ., Montreal, QC, Canada

Abstract: There are sex differences in schizophrenia and in the response to antipsychotic medication, with women exhibiting later onset, less severe symptoms, and better response to medication. Previous research suggests that estrogens may protect against the positive symptoms of schizophrenia, but is unclear if estrogens also protect against the cognitive symptoms of schizophrenia, such as deficits in reversal learning. This study investigated the effects of estradiol (E2) administered in conjunction with haloperidol (HAL), an antipsychotic medication known to ameliorate some deficits in reversal learning. For this experiment female Sprague Dawley rats were amphetamine (AMPH) sensitized to model the reversal learning deficits observed in schizophrenia. Rats were ovariectomized and either not administered E2 replacement, or administered a low chronic E2 replacement, or a high cyclic E2 replacement. A simple two lever operant box paradigm was used to assess reversal learning. Animals were trained to press one of two levers for a sucrose reward, and then the lever that delivered the reward was switched; reversal learning was measured as the latency to shift responding from the lever that was no longer rewarded, to the previously unrewarded lever. Latency to reverse lever pressing behavior was compared between rats that were administered saline (SAL) and rats that

were administered HAL in each of the three E2 replacement groups (no, low, and high). There was a main effect of drug, as HAL treated rats showed significantly shorter latency to reverse their behavior. There was no significant difference in reversal learning between SAL and HAL treated rats in the no E2 condition, however, in the low E2 condition HAL treated rats had significantly shorter latency to reverse their behavior. In the high cyclic E2 condition there was no significant difference between HAL and SAL treated rats. These findings demonstrate that HAL ameliorates reversal learning deficits induced by AMPH sensitization, and that a low chronic E2 replacement regime facilitates the effects of HAL on reversal learning.

Disclosures: **A. Almey:** None. **W.G. Brake:** None. **J. Oliel:** None. **L. Arena:** None.

Poster
(Unable to Attend)

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.20/PP12

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR 102568.

Title: Differential effects of androgens and sex on adult neurogenesis in the dentate gyrus of aged male and female rats

Authors: ***D. K. HAMSON**[1], S. R. WAINWRIGHT[2], C. CHOW[1], J. F. LALANZA[3], D. T. SAMUEL[1], N. V. WATSON[4], L. A. M. GALEA[1]
[1]Psychology, [2]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada; [3]UNIVERSITAT AUTÒNOMA DE BARCELONA, Barcelona, Spain; [4]Psychology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: The production and survival of neurons in the dentate gyrus of the adult hippocampus is severely decreased in aged rodents. The decline in neurogenesis is thought to be a contributing factor to age related cognitive impairment during normal senescence, but may contribute to neurodegenerative diseases such as Alzheimer's. We recently reported that the androgen, dihydrotestosterone, increased neurogenesis by modulating new neuron survival, but not cell proliferation, in young male rats via an androgen receptor dependent mechanism. In contrast, thirty days of androgen treatment did not affect neurogenesis in young female rats, suggesting androgens act in a sex dependent manner in young rodents. In order to understand if

dihydrotestosterone plays a role in regulating neurogenesis in aged rodents, we examined cell proliferation and neuron survival in 20 month old male and female Sprague Dawley rats. Males and females were gonadectomized, injected with bromodeoxyuridine (BrdU) to label new cells, and then treated with dihydrotestosterone for 30 days. Surprisingly, and in contrast to what we hypothesized, dihydrotestosterone decreased the number of BrdU positive cells in aged males, but increased the number of BrdU positive cells in aged females. Similar to our results in young rodents, androgen treatment did not affect Ki67 expression (an endogenous marker of cell proliferation) in both aged males and females, however, there was a sex difference in which aged females displayed fewer Ki67-expressing cells than males. The volume of the dentate gyrus was not affected by gonadectomy, androgen treatment, or sex. Together these data suggest androgens affect the number of cells in the dentate gyrus in a sex and age-dependent manner by promoting neurogenesis in young males and older females. The influence of both age and sex on the androgen-mediated alteration of adult hippocampal neurogenesis may have important implications for the treatment of neurodegenerative disease in both males and females.

Disclosures: D.K. Hamson: None. **S.R. Wainwright:** None. **C. Chow:** None. **J.F. Lalanza:** None. **D.T. Samuel:** None. **N.V. Watson:** None. **L.A.M. Galea:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

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Program#/Poster#: 451.21/PP13

Topic: E.03. Behavioral Neuroendocrinology

Support: University of Wisconsin-Milwaukee College of Letters and Sciences (KMF)

American Federation for Aging Research (AMF)

Title: Two cell-signaling mechanisms for one mnemonic outcome: How progesterone facilitates memory consolidation in the dorsal hippocampus

Authors: *A. M. FORTRESS, K. M. FRICK
Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: We previously showed in ovariectomized mice that intrahippocampal infusion of progesterone enhances novel object memory consolidation in a manner dependent on activation of dorsal hippocampal ERK and mTOR signaling. However, the role of membrane and

intracellular progesterone receptors (PRs) in mediating the effects of progesterone on memory consolidation and hippocampal cell signaling are unknown. Therefore, the goals of the present study were to: 1) investigate the roles of different progesterone receptors (PRs) in mediating hippocampal memory consolidation, and 2) identify downstream cell signaling pathways necessary for the mnemonic effects of PRs. The role of membrane-bound PRs was tested using bovine serum albumin-conjugated progesterone (BSA-P), and the role of intracellular PRs (PRA, PRB) was tested using the intracellular PR agonist R5020. Ovariectomized C57BL/6 female mice (10 weeks of age) were trained in a hippocampal-dependent novel object recognition task and were then infused into the dorsal hippocampus with vehicle, progesterone, BSA-P, or R5020 immediately afterwards. Memory was tested 48 h later. Two weeks later, mice were infused again and the dorsal hippocampus collected 5 min later for Western blotting of cell signaling proteins. Progesterone, BSA-P, and R5020 all enhanced novel object recognition memory consolidation. However, only progesterone and BSA-P activated ERK and mTOR signaling. Furthermore, dorsal hippocampal infusion of the ERK inhibitor U0126 blocked the memory-enhancing effects of BSA-P, but not R5020. These data suggest that the ability of membrane PRs, but not intracellular PRs, to enhance memory consolidation depends on rapid ERK and mTOR signaling. Interestingly, progesterone robustly activated canonical Wnt signaling in the dorsal hippocampus 5 min after infusion, which is consistent with our recent findings that canonical Wnt signaling is necessary for object recognition memory consolidation. R5020, but not BSA-P, also elicited a modest increase in canonical Wnt signaling. These data suggest a role for activation of canonical Wnt signaling in the memory-enhancing effects of intracellular PRs. Collectively, this study provides the first evidence illustrating that membrane and intracellular PRs may employ different molecular mechanisms to enhance hippocampal memory.

Disclosures: **A.M. Fortress:** None. **K.M. Frick:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.22/PP14

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01AG041372

State of Louisiana Board of Regents Graduate Fellowship LEQSF (2013-2018)GF-17

Title: GnRH attenuates the detrimental effects of antagonism of G protein-estrogen receptor (GPER) on memory performance of middle-aged ovariectomized rats

Authors: *J. DARLING[1], G. PEARL[1], Y. SAKAMOTO[1], J. M. DANIEL[1,2]
[1]Neurosci. Program, [2]Psychology, Tulane Univ., New Orleans, LA

Abstract: Gonadotropin releasing hormone (GnRH) administration improves hippocampal dependent memory in rodent models. Recent data from our lab and others suggest that this effect may be mediated by the ability of GnRH to increase production of neuroestradiol in the hippocampus. Neuroestradiol may act to impact memory via activation of classical estrogen receptors, ERα and ERβ, or through activation of the G protein-estrogen receptor GPER. The goal of the current experiment was to test the hypothesis that GnRH will attenuate detriments in performance on a hippocampal dependent radial arm maze task. Middle-aged rats were ovariectomized and then received 24 days of acquisition training on an eight-arm radial maze. After completion of training, half of the rats received daily subcutaneous injections of GnRH (1ml/kg) and half received daily injections of vehicle. After 15 days of injections, rats were tested on the radial maze to determine effects of the GPER antagonist, G15, on maze performance. Fifteen minutes prior to maze testing, rats received either a subcutaneous injection of G15 or vehicle. G15 (10μg/kg) significantly disrupted performance, as assessed by numbers of errors, in vehicle-treated rats but had no effect on GnRH-treated rats. Furthermore, the performance of GnRH and vehicle- and GnRH-treated rats were significantly different from each other after G15 administration. GnRH-treated rats made significantly fewer errors than vehicle rats when given a G15 dose of 10 μg/kg. Results indicate that GnRH attenuates the detrimental effects of G15 on memory performance of ovariectomized rats possibly through enhanced neuroestradiol production leading to activation of the GPCR GPER.

Disclosures: J. Darling: None. **G. Pearl:** None. **Y. Sakamoto:** None. **J.M. Daniel:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.23/PP15

Topic: E.03. Behavioral Neuroendocrinology

Title: Estradiol regulates dendrite length in the central nucleus of the amygdala in female rats

Authors: *L. M. FLANAGAN-CATO[1], S. L. FERRI[2], P. F. HILDEBRAND[3]
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Abstract: The ovarian hormone cycle is associated with a coordinated fluctuation in female behaviors, most notably, sexual behavior. Underlying these behavioral changes, estradiol and progesterone produce structural and neurochemical changes in the ventromedial nucleus of the hypothalamus, including changes in dendrite morphology, spine density, and the levels of AMPA-type glutamate receptor subunits, GluR1 and GluR2. Ovarian hormones induce other behavioral changes, such as reduced food and water intake, increased locomotor activity and changes in emotional behavior. The amygdala is a key structure for emotional learning and stress responses, and estradiol acts in the amygdala to reduce anxiety-like behavior. The possible effects of estradiol on dendrite structure in the amygdala, however, have not been described. We hypothesized that estradiol regulates dendrite morphology in the amygdala, with a focus on the central nucleus (CeA). Ovariectomized rats were treated with vehicle (sesame oil; n=4) or 17-β estradiol benzoate (EB; 10 μg in 100 μL sesame oil on two consecutive days; n=5). Animals were sacrificed 48 hours after the second EB injection. Brains were processed for Golgi impregnation, sectioned on a vibratome, and the sections were mounted onto microscope slides. CeA neurons were viewed at 100X and illustrated using camera lucida (5-6 neurons per animal). The locations of the neurons were identified as being within the medial or lateral CeA. The number of dendrites per neuron, dendrite length, and dendrite direction were evaluated using NIH Image 1.62. There were no significant differences between the treatment groups for the number of dendrites per neuron in either region (approximately 5 dendrites per neuron). The treatment groups also did not differ in the length of dendrites in the lateral zone of the CeA (182 versus 191 μm/dendrite). In the medial zone of the CeA, vehicle-treated animals had 35% shorter dendrites than the estradiol-treated group (145 + 9 versus 216 + 24 μm/dendrite; two-tailed t-test, $F(3,2)=10.63$, $p=0.0582$). These results suggest that estradiol specifically remodels the synaptic organization of the medial CeA. Given that the medial zone of the CeA plays a unique role in amygdalar function by coordinating the output of the amygdala complex to brain regions that control autonomic, endocrine and behavioral functions, it will be important for future studies to investigate the specific inputs to the medial CeA that are modulated by estradiol.

Disclosures: L.M. Flanagan-Cato: None. **S.L. Ferri:** None. **P.F. Hildebrand:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.24/PP16

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant AG0411374 to JMD

Title: Impact of acute IGF-1 administration on estradiol-regulated proteins in the hippocampus of ovariectomized rats: Implications for ligand-independent activation of estrogen receptors

Authors: *E. M. GRISSOM[1], J. M. DANIEL[1,2]
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Abstract: Short-term treatment with estradiol following ovariectomy (OVX) in aging rats results in long-term improvements in cognition and elevations in estradiol-associated proteins long after the estradiol treatment is terminated. Furthermore, these effects are blocked by antagonism of insulin-like growth factor 1 (IGF-1) receptors initiated at the time that estradiol treatment was terminated. In the absence of estradiol, IGF-1 can activate estrogen receptor-dependent transcription via ligand-independent pathways. Thus, we hypothesize that acute intracerebral ventricle (i.c.v.) administration of IGF-1 in OVX rats would increase estradiol-associated proteins transcribed by the estrogen response element (ERE). Female OVX'd rats were infused with IGF-1 in the right cerebral ventricle 24 h prior to tissue collection. The hippocampus was analyzed by western immunoblotting for the presence of the ERE-associated proteins ChAT, brain-derived neurotrophic factor (BDNF) and estrogen receptor alpha (ERα). Additionally, we also examined two proteins, post synaptic density 95 (PSD-95) and glial fibrillary acidic protein (GFAP) which are associated with fluctuations in estradiol but are transcribed by sequences other than the ERE. We found that acute i.c.v. administration of IGF-1 significantly elevated levels of ChAT, BDNF, and ERα. In contrast, there were no differences between IGF-1 and vehicle-treated rats in levels of PSD-95 or GFAP. Based on results of the current study, we find that IGF-1 administered i.c.v. is able to elevate levels of proteins transcribed by the ERE. These results suggest that binding of IGF-1 to its receptor can activate estradiol pathways and elevate levels of ChAT, BDNF, and ERα when estradiol is absent, lending further support to our model of ligand-independent activation of ERE transcription by IGF-1 in the absence of estradiol.

Disclosures: E.M. Grissom: None. **J.M. Daniel:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.25/PP17

Topic: E.03. Behavioral Neuroendocrinology

Title: The effect of time of day on musk shrew sexual behavior

Authors: K. VASILOFF, K. KELLY, *L. M. FREEMAN
Mary Baldwin Col., Staunton, VA

Abstract: Suncus murinus, also known as the Asian musk shrew, is a model species for studies of sexual behavior. The unique mating behaviors of musk shrews are due to the fact that the female is the aggressor. Initial mating practice begins with female lunges towards the male. Instead of the female performing lordosis, she instead tail wags to give indication of sexual receptiveness. The male will then proceed to mount the female until ejaculation. One of the advantages of using musk shrews includes that musk shrews do not have behavioral hormonal estrus cycles. Female musk shrews also only have a total gestation period of 30 days. Though this process has been observed in research, the optimal time for mating has not been studied. In this experiment, we looked at the musk shrews' sexual behaviors and how they differed between morning and evening. Because previous laboratory matings have been performed in the evening, we expected to see more successful matings during that time. The results from this study can aid future matings to be performed at a time where breeding is more efficacious. To look at the effect of a multitude of variables on shrew sexual behavior, we observed a total of 14 female shrews and 14 male shrews. To control specifically for the differences of mating behavior we recorded time of day, humidity, successful mating counts, aggressive lunge counts [female aggression in the form of an audible shriek and physical approach], and whether or not the male ejaculated. The first observations were conducted as a baseline, looking at normal behavior during the morning and evening. The second set of observations had a total n of 10, with an n of 5 in both the morning mating and evening mating groups. Two-way chi square showed significance between ejaculation and morning or evening matings [$X2(1)=4.16$, $p<.05$]. There was no significant difference between the amount of lunge counts and time of day, nor was there any significant difference between humidity and ejaculation. This suggests that the optimal time to mate shrews may be at the beginning of their light cycle, rather than at the end as previously predicted.

Disclosures: K. Vasiloff: None. **K. Kelly:** None. **L.M. Freeman:** None.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.26/PP18

Topic: E.03. Behavioral Neuroendocrinology

Title: Sociosexual behaviors during the transition from non-receptivity to receptivity in rats housed in a seminatural environment

Authors: *X. CHU, A. ÅGMO
Univ. of Tromsø, Tromso, Norway

Abstract: Behavioral estrus in intact, cycling female rats can be defined as the period between the first lordosis displayed during the estrus cycle and the lordosis that is not followed by another one within 60 minutes. In a seminatural environment with several males and females, an estrous female consistently displays lordosis in response to every male mount from the start of behavioral estrus until the end of it. This means that the female suddenly changes from a state of complete non-receptivity to a state of full receptivity and then abruptly changes back to non-receptivity. The mechanisms behind this swift receptivity change remain unclear. Here we present the results of a detailed study of sociosexual behaviors during the transition from non-receptivity to receptivity and vice versa. A preestrus phase was defined as a period before the initial lordosis and the postestrus phase was the period following the final lordosis. The duration of the preestrus and postestrus phases analyzed here was 5% of the length of estrus. Behaviors during these phases were compared to those observed during the first and last 5 % of behavioral estrus. The frequency of male mounting of the female was close to 0 both before and after estrus. It remained at a constant, high level throughout the period of estrus. The duration of the female's paracopulatory behaviors and of the males' pursuit of the female changed drastically from a very low level before estrus to a high level in estrus. It was strongly reduced in the postestrus phase. The female sniffed, anogenitally sniffed and pursued males equally before, during and after estrus. Avoiding behaviors such as fleeing from the male, rejection and nose-off did not change when the female entered into or went out of estrus. These data show that the main changes occurring when the female enters into behavioral estrus do not occur in female but in male behavior. The males pursue the female a lot more, and they start to mount her. Likewise, at the end of estrus they do no longer pursue the female and they don't mount her. Since the paracopulatory behaviors mainly are responses to the males' approaches, they increase and decrease in parallel with the males' behavior. It appears that the female becomes attractive to the male only when she is in a state of full receptivity. In fact, there are data showing that receptivity requires less estrogen than attractivity. The important role of the male in the determination of the duration of the female's behavioral estrus could not have been detected in a short mating test in a small cage housing a pair of rats. This is another example of the utility of seminatural environments for understanding the intricacies of sexual behaviors.

Disclosures: **X. Chu:** A. Employment/Salary (full or part-time):; University of Tromsø. **A. Ågmo:** A. Employment/Salary (full or part-time):; University of Tromsø.

Poster

451. Behavioral and Neural Effects of Gonadal Hormones

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 451.27/PP19

Topic: E.03. Behavioral Neuroendocrinology

Support: Cummings School of Veterinary Medicine at Tufts University

Title: Stress hyporesponsiveness during pregnancy and lactation in the rat: A role for the intestinal microbiota?

Authors: *P. E. MANN[1], R. S. BRIDGES[1], K. HUYNH[2], G. WIDMER[2]
[1]Dept Biomed Sci., [2]Dept Infectious Dis. and Global Hlth., Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA

Abstract: Rats, as women, normally display reduced anxiety and a reduced physiological reaction to stress during late pregnancy and lactation. Stress hyporesponsiveness has been traditionally thought to be due to the changes in the hypothalamic-pituitary-adrenal (HPA) axis caused by the physiological challenges of pregnancy and lactation. This reduced responsiveness to stress is considered an adaptive strategy to protect the fetus from exposure to high levels of glucocorticoids. An interesting, relatively new, avenue of research on stress responsiveness involves the role of the intestinal microbiota. Stress can change the composition of the microbiota, and conversely, the microbiota can change an animal's responsiveness to stress. The objective of this study was to determine, first, if the intestinal microbiota of rats changes during pregnancy and lactation as it does in women (Koren et al, 2012) and, second, what effects a high fat diet has on both stress hyporesponsiveness and the intestinal microbiota. Age-matched virgin (nulliparous), pregnant (primigravid) and lactating (primiparous) female Sprague-Dawley rats were fed either a normal diet (Harlan, Teklad, 2014, 4% fat) or a high-fat diet (Harlan, TD.06414, 60% fat). Ten days after the start of the high-fat diet, nulliparous females were placed in an activity chamber for 24 h and then tested on the elevated plus maze (EPM). Immediately after the EPM, the rats were euthanized and their brains collected. The pregnant animals were placed on the high-fat diet on day 1 of pregnancy (sperm present in the vaginal lavage). On day 5 of lactation the females were placed in the activity chamber (24 h) and then tested on the EPM and euthanized. Fecal pellets were collected periodically from all subjects after the start of the

high-fat diet. There were no differences in either activity or performance on the EPM in the nulliparous females. During lactation, there were no significant changes in activity between the high-fat diet females and controls. However, on the EPM, the high-fat diet females tended to show less anxiety-like behavior. Bacterial populations were analyzed using 16s rRNA amplicon sequencing (Illumina MiSeq) and were compared using the Unifrac metric. The microbiome of the animals was significantly clustered among cycling, pregnant and lactating animals particularly in the high-fat group (n=14). These preliminary findings indicate that the microbiome of the rat is modified by diet, as well as pregnancy and lactation. The pregnant and lactating rat may provide a useful model to examine stress hyporesponsiveness and its possible relationship to the intestinal microbiota both normally and in cases of maternal obesity.

Disclosures: **P.E. Mann:** None. **R.S. Bridges:** None. **K. Huynh:** None. **G. Widmer:** None.

Poster

452. HPA Axis

Location: Halls A-C

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Program#/Poster#: 452.01/PP20

Topic: E.05. Stress and the Brain

Support: FAPESP/Brazil

CAPES/Brazil

CNPq/Brazil

Title: The estrogen action on response of the hypothalamic-pituitary-adrenal axis to the hemorrhagic stress is not modulated by oxytocin

Authors: ***P. C. BARCELLOS-FILHO**, L. M. S. ALVES, G. A. A. TRASLAVIÑA, C. R. FRANCI
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Abstract: Oxytocin (OT) attenuates the activity of the stress system for specific stressors. It modulates the action of the hypothalamic-pituitary-adrenal (HPA) axis decreasing the secretion of ACTH and glucocorticoids. Estrogen also interferes in the activity of the HPA axis modulating the secretion of glucocorticoids in both basal and stress conditions. Whereas both

estrogen and OT may modulate stress responses, we aimed assess whether the HPA axis response to the hemorrhagic stress is modulated for an interaction between OT and estrogen. Estrogen-primed and unprimed ovariectomized female rats were subjected to the intracerebroventricular (icv) microinjection of OT antagonist (OTa) or NaCl 0,9% in the normovolemia or hemorrhage condition. Four blood samples were withdrawn immediately before (baseline) and 30, 60, 120 minutes after icv microinjections. All of the animals received a replacement of the same volume with isotonic saline after baseline blood withdrawn, but only control animals received replacement for other times. Plasma corticosterone and progesterone were measured for radioimmunoassay. The brains were removed after the end of experiment and processed for CRH and FOS protein immunohistochemistry. The secretion of corticosterone was altered by hemorrhage, hormonal treatment, and OTa. The post-hoc test showed that hemorrhage increased the secretion of corticosterone at 30 min (235.7 ± 27.6 ng / ml) and 120 min (202.2 ± 19.4 ng / mL) in rats treated with oil/icv OTa compared to the controls (129.4 ± 17.1 and 75.1 ± 17.0 ng/mL, respectively). Hemorrhage and hormonal treatment, but not OTa increased progesterone secretion. However, the interaction OTa with hemorrhage increased the secretion of progesterone. The post-hoc test revealed significant differences at 30 minutes (38.7 ± 13.1 ng / mL) and 60 minutes (43.9 ± 11.9 ng / mL) of hemorrhage in rats treated with oil/ icv OTa compared to the controls (13 ± 2,4 and 19,2 ± 4,9 ng / mL, respectively). Only the hemorrhage increased CRH/FOS immunoreactivity in the PVN neurons (9,33 ± 1,03) in comparison to the control (3,92 ± 0,61). The results indicate that the action of estrogen on the HPA axis activity is not modulated by oxytocin.

Disclosures: **P.C. Barcellos-Filho:** None. **L.M.S. Alves:** None. **G.A.A. Traslaviña:** None. **C.R. Franci:** None.

Poster

452. HPA Axis

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Topic: E.05. Stress and the Brain

Support: NIH NIDDK R01 DK091425 (YMU)

NIH NIDDK K01 DK078906 (YMU)

NIH NIDDK T32 DK059803 (AEBP)

Title: Ketogenic diet induces over-activity of the HPA axis: Necessity and sufficiency of metabolic ketosis

Authors: *A. E. BRUESTLE PACKARD[1], K. K. RYAN[2], K. HALCOMB[1], R. J. SEELEY[2], Y. M. ULRICH-LAI[1]
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Abstract: An individual's response to stress is profoundly influenced by their diet and metabolic state. Low-carbohydrate, high-fat diets (i.e., ketogenic diets; KD) induce ketosis, a process by which the liver converts fatty acids to ketone bodies providing an alternative fuel for the brain in the face of limited glucose availability. We hypothesized that metabolic ketosis potentiates HPA axis activation in order to enhance stress-induced fuel mobilization, thereby ensuring adequate fuel availability during stress despite low glucose. To that end, adult male Long-Evans rats were given ad libitum access to either low-carbohydrate KD or normal chow (controls), and the HPA and cfos responses to restraint stress were assessed. As expected, KD-fed rats had elevated plasma β-hydroxybutyrate, and gained less body weight than chow-fed controls despite consuming equivalent or greater calories, demonstrating the ability of the KD to induce ketosis. KD also increased basal and post-restraint plasma corticosterone, and elevated post-restraint cfos immunolabeling in stress-regulatory brain regions. Further, carbohydrate supplementation to KD-fed rats reversed the ketosis and prevented the HPA over-activity, suggesting that ketosis is necessary for the KD effects. Lastly, acute oral gavage of medium chain triglycerides to chow-fed rats induced ketosis and was sufficient to replicate the HPA effects, suggesting that ketosis is sufficient for HPA activation. Taken together, these results suggest that ketosis potentiates the HPA axis, and supports the concept that stress responses are influenced by metabolic status.

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Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.03/PP22

Topic: E.05. Stress and the Brain

Support: Syngenta Crop Protection LLC

Animal Health and Disease Research

Title: Atrazine activation of the hypothalamic-pituitary-adrenal axis

Authors: ***C. D. FORADORI**[1], R. J. KEMPPAINEN[1], A. D. ZIMMERMAN[1], M. A. JONES[1], K. YI[2], C. B. BRECKENRIDGE[2], L. R. HINDS[3], J. E. HEALY[3], R. J. HANDA[3]
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Abstract: Atrazine (ATR) is a commonly used pre-emergence/early post-emergence herbicide. ATR treatment for four days via gavage results in a decrease in luteinizing hormone (LH) pulse frequency. The mechanism by which this inhibition occurs is unknown. However, ATR treatment also leads to a dose dependent rise in plasma adrenocorticotropin hormone (ACTH) levels orginating from the anterior pituitary resulting in a subsequent release of corticosterone (CORT) and progesterone (P) from the adrenal gland. Similar increases in plasma CORT and/or P levels have been shown to reduce LH pulsatilty. We have subsequently demonstrated that if the adrenal gland is removed, ATR can no longer inhibit pulsatile LH release in rats. This has lead to the investigation of ATR activation of the hypothalamic-pituitary-adrenal (HPA) axis. Our current findings demonstrate that while ATR treatment results in a rise in ACTH levels, the same doses fail to induce a marker of neuronal activation (cFOS) in neurons of the paraventricular nucleus of the hypothalamus (PVN). In addition, neither rats lacking a pituitary nor animals treated with the synthetic glucocorticoid, dexamethasone, display increased CORT after ATR treatment, suggesting that ATR is stimulating the pituitary (corticotrophs) directly and not causing a release of corticotrophin releasing hormone (CRH) from the brain or directly stimulating the adrenal gland. To test this hypothesis, we treated immortalized murine corticotrophs (AtT-20) and *ex vivo* rat pituitary cultures with ATR or one of its major metabolites (diaminochlorotriazine, DACT; desethylatrazine, DEA or deisopropylatrazine, DIA) at concentrations of 0.0001, 0.001, 0.01, 0.10, 1.0 or 10 μM for 30 mins, then assayed for ACTH release. Neither ATR or any of its metabolites induced an increase in ACTH secretion at any dose nor did they potentiate ACTH release in conjunction with CRH. We then attempted to block the *in vivo* ATR-induce rise in ACTH with pre-treatment with one of two CRH receptor antagonists (antalarmin-30mg/kg or astressin-30μg/kg). Both antagonists blocked the stress or CRH induced rise in ACTH; however, only pre-treatment with astressin blocked the ATR induced rise in ACTH. These findings suggest ATR maybe working centrally to induce CRH release without activating a cellular pathway, resulting in somal cfos expression in CRH neurons of the of the PVN or CRH receptor 1 activation on corticotrophs.

Disclosures: **C.D. Foradori:** 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.; Syngenta Crop Protection LLC. **R.J. Kemppainen:** None. **A.D. Zimmerman:** None. **M.A. Jones:** None. **K. Yi:** A. Employment/Salary (full or part-time):; Syngenta Crop Protection LLC. **C.B. Breckenridge:** A. Employment/Salary (full or part-time):; Syngenta Crop Protection LLC. **L.R.**

Hinds: None. **J.E. Healy:** None. **R.J. Handa:** 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.; Syngenta Crop Protection LLC.

Poster

452. HPA Axis

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Program#/Poster#: 452.04/PP23

Topic: E.05. Stress and the Brain

Support: NIH Grant MH069860

AHA Grant 13PRE17100141

Title: Role of central glucagon-like peptide-1 in stress excitation

Authors: *S. GHOSAL[1], P. MAHBOD[2], J. M. MCKLVEEN[1], E. P. SMITH[2], R. J. SEELEY[2], D. A. D'ALESSIO[2], J. P. HERMAN[1]
[2]Intrnl. Med., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: Glucagon-like peptide-1(GLP-1) has widespread actions on central regulation of homeostatic function, including regulation of neuroendocrine responses to stress. In brain, GLP-1 expression is limited to discrete sets of neurons in the nucleus of solitary tract (NTS) and ventrolateral medulla. The GLP-1 receptor (*Glp1r*) has a wider expression profile in the CNS, and, in particular, is found in abundance in the hypothalamic paraventricular nucleus (PVN), which is the primary central activator of the hypothalamo-pituitary-adrenal (HPA) axis stress response. Here, we tested the hypothesis central GLP-1 signaling is necessary for stress excitation. We used the Cre/lox system to knockdown *Glp1r* in the PVN (crossing *Glp1r* $^{flox/flox}$ mice with *Sim1*-Cre mice). *Sim1* (single-minded 1) is a transcription factor and is expressed in the PVN, supraoptic nucleus, and medial basal amygdala. The distribution of *Sim1* and *Glp1r* expression overlap in the PVN, suggesting that any observed physiological or behavioral outcomes are due to PVN knockdown (KD). After confirmation of PVN KD of *Glp1r*, adult male Sim1-Cre; *Glp1r* $^{lox/flox}$ and littermate *Glp1r* $^{flox/flox}$ mice were subjected to (i) an acute psychogenic (restraint) stress, (ii) an acute systemic stress (exposure to hypoxia for 30 min), or (iii) chronic variable stress (CVS) for 2 weeks. Knockdown of *Glp1r* in the PVN did not alter body weight, food intake, or basal corticosterone release. However, loss of PVN *Glp1r* decreased

corticosterone responses to acute and chronic stress, and protected against chronic stress-induced attenuation of body weight gain. Loss of *Glp1r* also reduced anxiety-like behavior in the elevated plus maze test. No effects were observed in *Sim1*-Cre mice, indicated that effects are specific to *Glp1r* KD. Collectively, these data demonstrate that GLP-1 signaling in the PVN plays an important physiological role in both acute and long-term regulation of stress responses and is essential for driving responses to stress. Thus, GLP-1 therapeutics may prove useful for disorders linked to neuroendocrine hypofunction, such as posttraumatic stress disorder PTSD.

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Poster

452. HPA Axis

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Topic: E.05. Stress and the Brain

Support: NIMN intramural program project 1Z1AMH002386 (L.E.E.)

Title: Role of PACAP and its receptors in the mouse stress response determined by knockout phenocopy experiments

Authors: *Z. JIANG[1], I. THISTLETHWAITE[1], E. WEIHE[2], T. MUSTAFA[1], L. E. EIDEN[1]
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Abstract: PACAP acts at synapses in the central and peripheral nervous system to mediate hypothalamo-pituitary-adrenal and hypothalamo-sympatho-adrenal responses to various stressors (Stroth et al., *Ann. N.Y. Acad. Sci.* **1220**: 49, 2011). We and others have established some salient features of this regulation in the mouse, via comparison of wild-type and PACAP-deficient mice (Stroth and Eiden, *Neuroscience* **165**: 1025, 2010; Tsukiyama et al., *Stress* 14: 368, 2011; Stroth et al., *Endocrinology* **154**: 330, 2013). It appears that PACAP neurotransmission is required for epinephrine release from chromaffin cells caused by either systemic or psychogenic stressors, while PACAP neurotransmission is required for CORT elevation caused by psychogenic, but not systemic, stressors (Stroth et al., ibid; Tsukiyama et al., ibid; Lehmann et al., *Psychoneuroendocrinology* **38**: 702, 2013). Furthermore, depressive and anxiogenic effects of chronic psychogenic (social defeat) stress is greatly attenuated in PACAP-deficient mice,

suggesting PACAP receptor antagonism as a drug target in depression and other stress-mediated neuropsychiatric disorders (Lehmann et al., ibid). However, the PACAP-deficient phenotype has yet to be phenocopied in mice deficient for each of the three receptors thought to mediate PACAP's actions, including PAC1, VPAC1 and VPAC2. We have compared neurochemical and behavioral responses to PACAP and PAC1 deficiency using wild-type and PACAP- and PAC1-deficient C57Bl/6 male 2.5-3.5-month old mice. Acute (3 hr) restraint causes elevation of CORT (measured immediately after cessation of restraint) in wild-type C57Bl/6 mice and this increase is significantly attenuated in both PACAP- and PAC1-deficient mice. Continuous (2 hr/day) restraint causes sustained elevation of serum CORT (measured immediately after cessation of restraint) and weight loss, due to decreased food intake, in wild-type mice for up to 21 days. Weight loss and CORT elevation due to restraint are not observed in PACAP-deficient mice from day 7-21 of restraint. In contrast, weight loss and CORT elevation do occur throughout chronic restraint in PAC1-deficient mice, and are of similar magnitude to those observed in wild-type mice. We conclude that PACAP release, and PAC1 receptor stimulation, are required for HPA axis stimulation following acute psychogenic stress, while PACAP release, and stimulation of PACAP receptors in addition to PAC1, are required for continuous HPA axis stimulation during chronic psychogenic stress.

Disclosures: Z. Jiang: None. **I. Thistlethwaite:** None. **E. Weihe:** None. **T. Mustafa:** None. **L.E. Eiden:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.06/QQ1

Topic: E.05. Stress and the Brain

Support: Highlands Biological Station Grant in Aid of Research

Title: Effect of reproductive condition on the hypothalamic-pituitary-adrenal axis of a terrestrial salamander

Authors: *J. R. THOMAS, S. K. WOODLEY
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Abstract: In vertebrates, many responses to stressors are mediated by the hypothalamic-pituitary-adrenal (HPA) axis, of which corticotropin-releasing factor (CRF) and glucocorticoids

(GCs) such as corticosterone (CORT) are key players. The axis is sensitive to a variety of factors, and many species exhibit seasonal changes in both baseline and stress-induced GCs with levels being elevated during the breeding season (Romero, 2002). One factor thought to play a role in regulating the HPA axis is reproductive condition. To examine the effects of reproductive condition on the HPA axis, we conducted a field study using female red-legged salamanders (*Plethodon shermani*) which oviposit every other year, allowing comparison of reproductive and nonreproductive females under similar environmental conditions. We evaluated baseline and handling-induced levels of plasma CORT as well as the number and distribution of immunoreactive (IR) CRF neurons in both reproductive and nonreproductive females. In previous work, we identified five populations of CRF neurons in female *P. shermani*: subpallial amygdala, magnocellular preoptic area (POA), parvocellular ventral POA, hypothalamus, and locus coeruleus (LC). Of these populations, those cells found in the POA, analogous to the mammalian paraventricular nucleus (PVN), are implicated in the HPA axis. We hypothesized that reproductive condition would modulate the HPA axis, with reproductive females having elevated baseline plasma CORT, blunted CORT responses to handling stress, and fewer CRF-IR neurons in the POA. We saw no differences in baseline plasma CORT, and neither reproductive nor nonreproductive females had CORT responses to handling. The total number of CRF-IR neurons was significantly higher in nonreproductive compared to reproductive females when sampled under baseline conditions, due in part to differences in the LC, an area of the brain involved in activation of the sympathetic nervous system (SNS). While no effect of reproductive condition on plasma CORT or CRF-IR neurons in the POA was observed, our results suggest that reproductive condition may affect CRF in its role as a neurotransmitter in the SNS and the activation of the locus coeruleus norepinephrine (LC-NE) system.

Disclosures: **J.R. Thomas:** None. **S.K. Woodley:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.07/QQ2

Topic: E.05. Stress and the Brain

Support: NIH Grant R01 DK091425

NIH Grant T32 NS007453

Title: Stress system activation by a natural reward: Responses to novel and repeated sucrose intake

Authors: *A. E. EGAN, Y. M. ULRICH-LAI
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Abstract: Pharmacological rewards such as drugs of abuse evoke robust stress responses, including activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis. However, it is not known to what extent natural rewards (e.g., palatable foods) elicit comparable stress responses. In order to address this question, physiological stress responses (HPA axis hormones; heart rate) and neuronal activation (pCREB immunolabeling) to novel or repeated intake of sucrose were measured. Adult, male Long-Evans rats with *ad libitum* food and water were given either a single (day 1) or repeated (twice-daily for 14 days) limited exposure to a second drink sipper containing sucrose (4 ml of 30% sucrose or water control for up to 30 min). Sucrose-fed rats consistently drank more than water-fed controls, suggesting that sucrose is highly palatable to rats. Stress responses on day 1 (plasma corticosterone, heart rate) were markedly increased after presentation of the second sipper regardless of drink type. After repeated exposure, the plasma corticosterone response habituated to similar extents in both the water and sucrose groups, and post-sipper pCREB immunolabeling in the hypothalamic paraventricular nucleus (PVN) did not vary with drink type. In contrast, the heart rate response habituated more after water than sucrose. Taken together, these data suggest that the stress responses occurred primarily in response to the sipper presentation itself (as opposed to the reward value of the offered drink). This work supports the hypothesis that natural rewards (like palatable food intake) do not inherently activate stress responses, but rather stress activation results primarily from unfamiliar interventions by investigators.

Disclosures: A.E. Egan: None. **Y.M. Ulrich-Lai:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.08/QQ3

Topic: E.05. Stress and the Brain

Support: Univ Adelaide

Title: The impact of toll-like receptor 4 on the hypothalamus ~ pituitary ~ adrenal axis structure and function

Authors: *M. R. HUTCHINSON[1], J. LIU[1], F. BUISMAN-PIJLMAN[2]
[1]Discipline of Physiology, Sch. of Med. Sci., [2]Discipline of Pharmacology, Sch. of Med. Sci., Univ. Adelaide, Adelaide, Australia

Abstract: Toll-like receptor 4 (TLR4) is part of the innate immune system, and TLR4 activation triggers the Hypothalamus-Pituitary-Adrenal (HPA) axis. Genetic knockout of TLR4 results in a hyporesponsive HPA axis to immune stress, but little is known about TLR4 involvement in the psychological stress response even though a dysregulation of both immune and HPA systems is evident in multiple psychological disorders. The current study aims to investigate the role TLR4 plays in HPA modulation by characterising strain differences between TLR4 genetic knockout (KO) and wild type Balb/c (WT) mice in their HPA anatomy, behavioural and HPA responses to forced swim stress and direct adrenal activation. Methods: To stimulate the HPA axis, WT and KO mice (n= 6-8 in each group) were given either Forced-swim stress or administered adrenocorticotrophic hormone (ACTH). Non-stressed or vehicle groups were also used as respective controls. Following acute activation, blood serum, hippocampus and hypothalamus samples were harvested. Serum samples were analysed for circulating Corticosterone, ACTH and corticosteroid binding globulin (CBG) levels, while brain expression of CBG were measured. Naive WT and KO animals were used to investigate strain differences in adrenal cortex size. Results: KO mice display smaller adrenal cortex size ($p< 0.05$), and a corresponding lower level of baseline ($p< 0.05$) and post stress ($p<0.05$) circulating corticosterone concentration, but higher ACTH ($p<0.001$) and CBG ($p<0.001$) expression. Higher levels of CBG expression were also evident in the hippocampus ($p< 0.01$) but no strain difference was found in the hypothalamus. However, a maximal dose of ACTH to directly activate the adrenal glands revealed that KO and WT adrenals were able to achieve the similar corticosterone output. Conclusion: Genetic KO of TLR4 influences HPA activity on a fundamental level, but this difference is likely to lie in the hippocampus, hypothalamus or pituitary (HP) structures rather than in adrenal function. Modification of TLR4 functioning could therefore lead to adaptations in the HPA axis, potentially having implications for the multiple systems that are influenced by HPA functions such as metabolism and immune functioning.

Disclosures: **M.R. Hutchinson:** None. **J. Liu:** None. **F. Buisman-Pijlman:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.09/QQ4

Topic: E.05. Stress and the Brain

Support: CIHR 86501

Title: Hypothalamic microcircuits responsible for stereotyped behavior following stress

Authors: *T. FÜZESI, J. I. WAMSTEEKER CUSULIN, W. INOUE, J. S. BAINS
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Abstract: To survive in a changing environment requires appropriate threat assessment followed by the rapid engagement of neural and endocrine systems to initiate behavioural and visceral adaptations to meet the impending challenge. Corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) are critical for launching the visceral response to stress. Intriguingly the successful completion of this task is followed by series of discrete and stereotyped behaviours that may serve a disengagement function that allows neural circuits to re-set following stress. There is strong evidence that one these post-stress stereotyped behaviours, repetitive grooming, involves the hypothalamus; but whether it is explicitly linked to stress circuitry is not known. We used an approach that combines optogenetics, electrophysiology, anatomical tracing and behavior, to ask whether disengagement behaviours like grooming are mediated by microcircuits that are embedded within the larger circuit architecture of the stress response. Specifically, we focused on the CRH neurons in PVN, to determine their role in dis-engagement behaviour following stress. We utilized a transgenic CRH-Cre mouse line and targeted PVN CRH neurons with recombinant adeno-associated virus carrying channelrhodopsin 2. *In vivo* stimulation of PVN CRH neurons with blue light induced rapid and robust grooming. This was accompanied by an increase in c-fos expression in the perifornical area/ lateral hypothalamus (PfA/LH) and the lateral septum. In order to elucidate the neural circuit(s) responsible for stimulated grooming, we traced CRH fibers and identified connections to neurons in the PfA/LH, but no direct connections to the lateral septum. We then used whole-cell patch clamp recordings combined with light stimulation *in vitro* and determined that neurons in the PfA/LH receive direct glutamatergic input from PVN CRH neurons. To ask whether PVN CRH neurons are also necessary for disengagement behavior following stress we injected an AAV carrying the Archaerhodopsin T construct into the PVN. Subsequent inhibition of PVN CRH neurons with yellow light dramatically decreased the time spent grooming after exposure to stress. Our data provide evidence that PVN CRH neurons serve a dual function, contributing to both the launch of the visceral response to stress and driving behaviours that are associated with the termination of the stress response.

Disclosures: T. Füzesi: None. **J.I. Wamsteeker Cusulin:** None. **W. Inoue:** None. **J.S. Bains:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.10/QQ5

Topic: E.05. Stress and the Brain

Support: CIHR

HBI postdoctral fellowship

Title: Brainstem noradrenergic afferents excite hypothalamic neurons through glutamate co-release

Authors: *W. INOUE[1,2], T. FUZESI[3], D. BAIMOUKHAMETOVA[3], Q. PITTMAN[3], J. BAINS[3]
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Abstract: The paraventricular nucleus of the hypothalamus (PVN) integrates inputs from diverse stress-sensitive brain areas to regulate the hypothalamus-pituitary-adrenal axis. Tyrosine hydroxylase (TH)-expressing neurons in the caudal medulla densely innervate the PVN, release noradrenaline (NA), and increase the excitability of PVN neurons. Reports indicate that the effects of NA may not be direct and may require glutamate as an intermediary. Interestingly, subpopulations of caudal medulla TH neurons co-express vesicular glutamate transporter 2, raising the possibility that glutamate may be released as a co-transmitter from these fibres. To address this question, we used optogenetic and electrophysiological approaches. We stereotaxically injected Cre-recombinase-dependent adeno-associated viral vector (AAV) carrying channelrhodopsin 2 (ChR2)-enhanced yellow fluorescent protein (eYFP) into the caudal medulla of mice that express Cre under the control of TH promoter (TH-IRES-Cre mice). We observed that eYFP+ axons innervating the PVN were TH immunopositive, verifying targeted expression. Using whole-cell voltage clamp recordings from PVN neurons, blue light illumination (473 nm, 5 ms) evoked inward postsynaptic currents (PSCs) with short latency (3.7 ± 0.2 ms). The PSCs had multiple peaks (the largest peak averaged at -69 ± 7.4 pA). In current-clamp mode, a single light stimulation evoked a rapid postsynaptic depolarization, which occasionally generated an action potential, and a train of light (10 Hz for 1 s) generated a burst of action potentials. The light-evoked PSCs were unaffected by a GABAA receptor antagonist but

were completely abolished by ionotropic glutamate receptor blockade, indicating that that NAergic terminals in the PVN co-release glutamate. An alpha-1 adrenergic receptor antagonist (prazosin) moderately reduced the amplitude of PSCs, suggesting that NA tonically modulates the glutamate co-release in an autocrine/paracrine manner. These results support the hypothesis that glutamate/NA co-release synergistically increases the excitability of PVN neurons.

Disclosures: W. Inoue: None. **T. Fuzesi:** None. **D. Baimoukhametova:** None. **Q. Pittman:** None. **J. Bains:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.11/QQ6

Topic: E.05. Stress and the Brain

Support: 5K01MH096148-02

Title: New Aspect of Anxiety Behavior, a View from the PVN Crh

Authors: *R. ZHANG[1], M. ASAI[1,2], M. JOACHIM[1], Y. SHEN[1], C. B. SAPER[3], J. A. MAJZOUB[1]
[1]Medicine, Endocrinol., Childrens Hospital, Harvard Med. Sch., Boston, MA; [2]Dept. of Pathology, Nagoya Univ. Grad. Sch. of Med., Nagoya 466-8550, Japan; [3]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: The hypothalamic-pituitary-adrenal (HPA) system is the major pathway in the mediation of the stress response. The hypothalamus releases corticotropin-releasing hormone (Crh) in response to stress, which triggers the release of adrenocorticotropin (ACTH) on from the pituitary gland, subsequently causing corticosteroid release from the adrenal cortex. Despite the indisputable role of PVN Crh in the regulation of the HPA axis, the role of PVN Crh in anxiety behavior regulation remains largely unknown. We generated Crh flox (Crh fl/fl) and crossed these with Sim1-Cre mice to mainly delete Crh in the PVN (PVN CrhKO). PVN CrhKO mice have normal bodyweight, food intake and Shirpa-evaluated behaviors. We used PVN CrhKO mice to study:
1) Role of PVN Crh in HPA regulation:
Diurnal plasma glucocorticoid rhythm was investigated through tail bleeding in freely moved mice in their home cages. Both control (Crhfl/fl) and PVN CrhKO mice had a diurnal rhymn in

corticosterone (nadir in the early morning and, peak in the late afternoon. However, compared to control mice, loss of PVN Crh significantly decreased plasma corticosterone. When we tested stress-stimulated HPA responsivity using 30min restraint, plasma corticosterone increased rapidly in all mice, but the peak in PVN CrhKO was 2.6 fold less compared to the control group. These data confirm the role of PVN Crh in HPA regulation under both basal and stress conditions.
2) Role of PVN Crh in anxiety behavior regulation:
Anxiety behaviors were studied using open field, light-dark box and elevated plus maze (EPM) tests. We consistently found that disruption of PVN Crh reduced anxiety-like behaviors, including a) increased cumulative time/frequency in the center (open field) and open arms (EPM), and decreased latency to center (open field) and light box (light-dark). There were no differences for total distance moved or velocity of movement between the control and PVNCrhKO mice among all tests.
In summary, our data confirm that PVN Crh controls the HPA axis under basal and stress conditions. Our data also indicates that PVN Crh is involved in the regulation of anxiety-like behaviors, suggesting a region-specific function of Crh in the development of anxiety disorders.

Disclosures: **R. Zhang:** None. **M. Asai:** None. **M. Joachim:** None. **Y. Shen:** None. **C.B. Saper:** None. **J.A. Majzoub:** None.

Poster

452. HPA Axis

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.12/QQ7

Topic: E.05. Stress and the Brain

Support: NIH Grant MH59911

Title: Attenuated activation of glucagon-like peptide-1 (GLP-1)- and prolactin-releasing peptide (PrRP)-positive hindbrain neurons may contribute to fasting-mediated reductions in anxiety-like behavior and paraventricular hypothalamic (PVN) responses to cognitive stress

Authors: *J. MANISCALCO[1], P. J. GORDON[2], L. RINAMAN[2]
[2]Dept. of Neurosci., [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Prolonged food restriction/fasting reduces anxiety and central drive to the hypothalamic-pituitary-adrenal (HPA) stress axis in rats. However, mechanisms underlying the

link between metabolic state and stress responsiveness are unresolved. Based on their anatomical and functional properties, hindbrain GLP-1 and PrRP neurons are well positioned to integrate metabolic feedback with stress responses. We hypothesized that cognitive stress activates GLP-1 and PrRP neurons, and that overnight fasting attenuates their activation commensurate with reduced anxiety and reduced activation of neurons in the medial parvocellular PVN (mpPVN). To test this, adult male Sprague-Dawley rats were fed ad lib or were fasted overnight for 16-18 hr. Anxiety-like behavior on the elevated plus maze (EPMZ) was assessed in one cohort of ad lib fed vs. fasted rats. A second cohort of fed and fasted rats was perfused with fixative 90 min after 30-min restraint (RES) or 5-min elevated platform (EP) exposure. Fixed brains were sectioned and processed for dual immunocytochemical localization of nuclear cFos and cytoplasmic GLP-1 or PrRP. Similar to chronic food restriction, an overnight fast significantly reduced anxiety-like behavior on the EPMZ. In ad lib-fed rats, ~30% of GLP-1 and PrRP neurons expressed cFos under control conditions, whereas increased proportions of both neural populations expressed cFos after RES (GLP-1, 66%; PrRP, 90%) and EP (GLP-1, 75%; PrRP, 52%). In fasted rats, GLP-1 activation was nearly abolished under all conditions (0-1%). Fasting also nearly abolished PrRP activation at baseline and after EP stress (0-2%), and significantly reduced PrRP activation after RES (18%). Fasting produced non-significant trends towards decreased mpPVN cFos expression at baseline and after EP stress, but not after RES. Within the anterior ventral bed nucleus of the stria terminalis, a region that contributes to anxiety-like behavior, both RES and EP increased cFos activation in ad lib-fed rats, whereas activation was significantly attenuated in fasted rats. We conclude that an overnight fast is sufficient to 1) decrease anxiety-like behavior, 2) decrease cognitive stress-induced activation of hindbrain GLP-1 and PrRP neurons, and 3) decrease neural activation in hypothalamic and limbic forebrain regions associated with HPA axis activation and anxiety-like behavior. Metabolic modulation of GLP-1 and PrRP neuronal activation is a potential mechanism through which caloric deficit alters HPA function and anxiety-like behavior.

Disclosures: **J. Maniscalco:** None. **P.J. Gordon:** None. **L. Rinaman:** None.

Poster

452. HPA Axis

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 452.13/QQ8

Topic: E.05. Stress and the Brain

Support: NIH 2R01NS029728-18A1

Title: Hindbrain catecholaminergic projections to the paraventricular nucleus are required for activation of glutamatergic terminals by glycemic challenges

Authors: *C. S. JOHNSON, A. G. WATTS
USC, Los Angeles, CA

Abstract: Hindbrain catecholaminergic inputs to the paraventricular nucleus of the hypothalamus (PVH) are necessary for the full response of neuroendocrine neurons to glycemic challenges. The drive provided to the neuroendocrine neurons by ascending catecholaminergic afferents also appears to require a glutamatergic component, as direct norepinephrine stimulation of the peri-PVH region results in a significant increase in glutamatergic excitatory postsynaptic potentials. To determine if these hindbrain catecholaminergic afferents are required to increase the excitatory synaptic drive to neuroendocrine neurons in the medial parvocellular region (mp) of the PVH, we have developed an immunocytochemical (ICC) method to assess if appositions alter their activity in response to a stimulus. This method relies on detecting the increased phosphorylation states of two key intracellular signaling intermediaries, ERK and synapsin I (Syn I), that occur as terminals become activated. Adult male Sprague-Dawley rats received central injections of the immunotoxin saporin conjugated with a dopamine-β-hydroxylase antibody, aimed at the PVH, to ablate catecholaminergic projections from the hindbrain. Rats were then fitted with jugular catheters and administered 2U/kg/ml insulin or 250 mg/kg 2-deoxy-glucose. Following perfusion, coronal sections were cut through the PVH and run for ICC using antibodies against Vesicular Glutamate Transporter 2 (VGluT2), phospho-ERK, and phospho-Syn I. Confocal Z-stacked images through the PVH were acquired, and analysis of 3D images was performed using Volocity software to assess colocalization of VGluT2 with phospho-ERK & phospho-Syn I in terminals within the PVHmp. The mean Pearson's Colocalization Coefficient was compared across groups. With a glycemic challenge, animals with intact catecholaminergic projections showed an increased numbers of appositions exhibiting colocalization of VGluT2 with the phosphorylated signaling molecules compared to controls. Animals without hindbrain catecholaminergic projections, however, had significantly fewer colocalized appositions. This suggests that catecholaminergic inputs from the hindbrain to the PVH are necessary for the glutamatergic excitation to the neuroendocrine neurons in the medial parvocellular region of the PVH in response to a glycemic stressor, as demonstrated through changes in appositional activity levels.

Disclosures: C.S. Johnson: None. **A.G. Watts:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.01/QQ9

Topic: E.07. Food Intake and Energy Balance

Support: MEXT Grant-in-Aid for Scientific Research

Title: A high ratio of dietary lysine to tryptophan suppressed kynurenic acid production in rat brain

Authors: ***T. FUKUWATARI**, A. OKUNO, S. MATSUTANI, S. GOTO, K. SHIBATA
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Abstract: At endogenous brain concentrations, the tryptophan metabolite kynurenic acid (KYNA) is a preferential antagonist of the α7 nicotinic acetylcholine receptor. Animal experiments show that elevated KYNA levels reduce glutamate and dopamine (DA) levels, and contribute cognitive dysfunction. Patients with schizophrenia show elevated KYNA levels in the brain and cerebral spinal fluid, suggesting the involvement of KYNA in the pathophysiology of schizophrenia. We reported that high tryptophan diet increased KYNA production and reduced DA release in rat brain (J Neurochem, 118: 796, 2011). We also showed that high lysine diet rescued dietary tryptophan-induced DA turnover decrease via inhibition of KYNA production. In the present study, we investigated the associations between ratio of dietary lysine to tryptophan and KYNA production in rat brain. Rats were given 0.5% or 1.5% tryptophan added 20% casein diet with 1%, 2% or 3% lysine, or 0.5% tryptophan added 20% gluten diet with 1%, 2% or 3% lysine for 7 days. Concentrations of KYNA and KYNA's immediate bioprecursor kynurenine (KYN) were measured in the striatum. Supplementation of more than 1% lysine suppressed the brain KYNA increarse in rats fed with 0.5% tryptophan added 20% casein diet, more than 2% lysine did in rats with 0.5% tryptophan added 20% gluten diet, and supplementation of 3% lysine did in rats with 1.5% tryptophan added 20% casein diet. Brain KYN contents were not changed by lysine supplementation. The ratio of lysine to tryptophan (mol/mol) significantly correlated with inhibitory effects for KYNA production. Lysine/tryptophan ratios of more than 3.6 suppressed dietary tryptophan-induced KYNA production, but not less than 2.8. These results suggest that dietary combination of amino acids influence KYNA production in the brain. Dietary manipulation of KYNA formation in astrocytes may provide useful approach for the treatment of DA related disorders.

Disclosures: **T. Fukuwatari:** None. **A. Okuno:** None. **S. Matsutani:** None. **S. Goto:** None. **K. Shibata:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.02/QQ10

Topic: E.07. Food Intake and Energy Balance

Support: KAKENHI26860134

Title: Localization of FABP3 in the mouse cingulate cortex and its possible role in the regulation of inhibitory neurons

Authors: *Y. YAMAMOTO[1], K. SHARIFI[1], A. ISLAM[1], M. EBRAHIMI[1], Y. YASUMOTO[1], H. MIYAZAKI[1], Y. KAGAWA[1], T. SAWADA[1], N. TOKUDA[1], K. FUKUNAGA[2], Y. OWADA[1]
[1]Yamaguchi Univ., Ube, Japan; [2]Tohoku Univ., Sendai, Japan

Abstract: Polyunsaturated fatty acids (PUFAs) are important for higher brain functions and nutritional deficiency of PUFAs impacts emotional and cognitive function. We previously showed that fatty acid binding protein 3 (FABP3), an intracellular chaperon for PUFAs, was expressed in the developing and mature brain, and that FABP3 gene ablation in mice resulted in altered cognitive and emotional behaviors with the decrease of PUFA uptake into the brain. However, the precise localization of FABP3 in the cingulate cortex, which is one of the important brain regions for coordination of such behaviors, and the role of FABP3 in the neuronal plasticity control are still unknown. In this study, we immunohistochemically examined the detail localization of FABP3 in cingulate cortex. Approximately 80% of FABP3+ neurons in cingulate cortex were also positive for parvalbumin and GAD67, both of which are markers of inhibitory neurons, but not for vesicular glutamate transporter 1, a marker of excitatory neurons. In the cingulate cortex of FABP3 gene ablated mice, there is no difference in the number of parvalbumin+ and/or
GAD67+ neurons compared with wild-type mice. Interestingly, in western
blot analysis, a significant increase in the expression of GAD67 was detected in cingulate cortex of FABP3 gene ablated mice. In microdialysis analysis, basal GABA release was significantly increased in the cingulate contex, while glutamate release was significantly decreased. Taken together, a close relationship between FABP3 function and GABAergic neurotransmission was highly suggested, and it is possible that such imbalance between GABA and glutamate in cingulate cortex is closely associated with the abnormal behaviors seen in FABP3 gene ablated mice.

Disclosures: **Y. Yamamoto:** None. **K. Sharifi:** None. **A. Islam:** None. **M. Ebrahimi:** None. **Y. Yasumoto:** None. **H. Miyazaki:** None. **Y. Kagawa:** None. **T. Sawada:** None. **N. Tokuda:** None. **K. Fukunaga:** None. **Y. Owada:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.03/QQ11

Topic: E.07. Food Intake and Energy Balance

Support: DC009997 Ajinomoto USA.

Title: Effects of bariatric interventions on sweet reward seeking

Authors: ***W. HAN**[1,2], J. NIU[1,2], G. J. SCHWARTZ[3], I. E. DE ARAUJO[1,2]
[1]John B. Pierce Lab., New Haven, CT; [2]Dept Psychiatry Yale university, New Haven, CT; [3]Albert Einstein Col. of Med., Bronx, NY

Abstract: We show in mice that daily exposure to intake of sugar, but not to artificial sweeteners, leads to insensitivity to reward devaluation (habitual and compusive sweet reward seeking). Devaluation of sweet reward was achieved via either satiation, taste adulteration or conditioned aversion. Mice sustaining a bariatric procedure resulting in a bypass of the duodenum, however, did not develop habitual or compulsive sweet reward seeking. Consistently, duodenal infusions of glucose resulted in greater dopamine release in dorsal striatum compared to jejunal infusions. Conversely, optogenetic activation of direct pathway neurons of dorsal striatum was sufficient to restore habitual and compulsive sweet reward seeking in bypassed mice. We conclude that striatal dopamine signaling is critical for changes in sugar preferences that follow malabsorptive procedures.

Disclosures: **W. Han:** None. **J. Niu:** None. **I.E. de Araujo:** None. **G.J. Schwartz:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

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Program#/Poster#: 453.04/QQ12

Topic: E.07. Food Intake and Energy Balance

Support: University of Wisconsin – Eau Claire Faculty/Student Research Collaboration grant

Title: Pramipexole decreases the discriminative stimulus effects produced by 22 hours food deprivation

Authors: *M. A. VANDEN AVOND, M. A. BARLOW, C. A. TODDES, B. GOMER, B. BERTI, K. J. OLSON, A. R. JOHNSON, D. C. JEWETT
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Abstract: We have developed and refined a food-deprivation discrimination paradigm that may serve as an animal model of 'hunger'. We examined the ability of pramipexole, a D2/D3 agonist used clinically to treat Parkinson's disease and restless leg syndrome, to reduce the effects of acute food deprivation in rats trained to discriminate between 2 and 22 hrs food deprivation in an operant choice paradigm. Generalization testing began after the acute food deprivation discrimination was acquired (~90 daily sessions). Prior to cumulative dose-generalization tests, subjects were food deprived for 22 hrs. Injections of vehicle and pramipexole (0.001-0.032 mg/kg, s.c.) occurred every 35 minutes (a 30 min pretreatment time and a 5 min response period) until a complete pramipexole dose-effect function was generated. Food intake was measured for 1 hr after the generalization tests. Pramipexole (0.01 mg/kg) significantly reduced the discriminative stimulus effects of 22 hrs deprivation. Pramipexole (0.01-0.032 mg/kg) also significantly reduced response rates and post-session food consumption. These results are consistent with our previous research demonstrating amphetamine decreases the discriminative stimulus effects of 22 hrs food deprivation and support the hypothesis that dopamine mediates food consumption by mechanisms related to 'hunger.'

Disclosures: M.A. Vanden Avond: None. **M.A. Barlow:** None. **C.A. Toddes:** None. **B. Gomer:** None. **B. Berti:** None. **K.J. Olson:** None. **A.R. Johnson:** None. **D.C. Jewett:** None.

Poster

453. Monoamines and Other Regulators

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK083410

Title: AMPK signaling is associated with synphilin-1-induced obesity

Authors: T. LI[1], J. LIU[1], D. YANG[1], A. MOGHADAM[2], P. CHOI[2], X. LI[1], S. BI[2], T. H. MORAN[2], *W. SMITH[1]
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Abstract: Synphilin-1 is a cytoplasmic protein with enriched expression in neurons. Previously our studies suggest that synphilin-1 is involved in the regulation of energy homeostasis. Expression of synphilin-1 in neurons induces hyperphagia and obesity-like phenotypes in both *Drosophila* and mouse models. However, the mechanism through which synphilin-1 affects energy homeostasis remains unclear. Here we demonstrate that the expression of synphilin-1 increased AMPK phosphorylation. Moreover, synphilin-1 is associated with AMPK in co-expressed cells and in mouse brains. Knockdown of AMPK in transgenic flies expressing human synphilin-1 reduced food intake and prevented body weight gain. In human synphilin-1 transgenic mice, there was a significant increase in phosphorylation of AMPK in the hypothalamus. In normal non-transgenic mice, fasting increases and refeeding decreases hypothalamic AMPK phosphorylation. In contrast, fasting and refeeding has only slight effects on AMPK phosphorylation in synphilin-1 transgenic mice. Taken together, these findings provide a novel cellular function of synphilin-1 in the maintenance of energy homeostasis.

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: FAPESP

CNPq

CAPES

Title: CB1 receptors into the Prelimbic Cortex modulate food intake in fasted rats

Authors: *A. A. SCOPINHO[1], L. B. M. RESSTEL[2], F. M. A. CORRÊA[2]
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Abstract: INTRODUCTION: The endocannabinoid system is involved in the central regulation of feeding behavior. Systemic administration of exogenous cannabinoids or endocannabinoids stimulates eating, and systemic administration of CB1 antagonist attenuates agonists' stimulatory effects on food intake, suggesting the involvement of these receptors in food intake modulation. Although CB1 receptors are present principally in the central nervous system (CNS), little is known about which areas are involved in this modulation and the role of endocannabinoids system. Therefore, the aim of the present study is to investigate the effects evoked by CB1 receptors antagonist (AM251) microinjected in the prelimbic cortex (PL) on the food intake regulation. METHODS: CB1 receptor antagonist AM251 (10, 50, 100pmol/100nl) or aCSF-artificial cerebrospinal fluid were microinjected into the PL of fed or fasted Wistar rats and 10 min later, the food intake test was performed during one hour for food intake determination. RESULTS: The amount of food ingested by fasted animals was significantly higher than fed animals ($F(1, 48) = 482,2$; $P<0.001$). Moreover, significant effects of treatment with doses of AM251 ($F(3, 48) = 74,41$; $P<0.001$) and interaction between the two factors $F(3, 48) = 78,26$; $P<0.001$) for total food intake (n=6-8 each group) were observed only in fasted rats. CONCLUSION: The blockade of PL CB1 receptors inhibited food intake in fasted animals, suggesting that the PL endocannabinoid system modulated food intake behavior and satiation.
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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NSERC

CIHR

Title: Single rapamycin administration induces prolonged downward shift in defended body weight in rats

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Abstract: Manipulation of body weight set point may be an effective weight loss and maintenance strategy as the homeostatic mechanism governing energy balance remains intact even in obese conditions and counters the effort to lose weight. However, how the set point is determined is not well understood. We show that a single injection of rapamycin (RAP), an mTOR inhibitor, is sufficient to shift the set point in rats. Intraperitoneal RAP decreased food intake and daily weight gain for several days, but surprisingly, there was also a long-term reduction in body weight which lasted at least 10 weeks without additional RAP injection. These effects were not due to malaise or glucose intolerance. Two RAP administrations with a two-week interval had additive effects on body weight without desensitization and significantly reduced the white adipose tissue weight. When challenged with food deprivation, vehicle and RAP-treated rats responded with rebound hyperphagia, suggesting that RAP was not inhibiting compensatory responses to weight loss. Instead, RAP animals defended a lower body weight achieved after RAP treatment. Decreased food intake and body weight were also seen with intracerebroventricular injection of RAP, indicating that the RAP effect is at least partially mediated by the brain. In summary, we found a novel effect of RAP that maintains lower body weight by shifting the set point long-term. Thus, RAP and related compounds may be unique tools to investigate the mechanisms by which the defended level of body weight is determined; such compounds may also be used to complement weight loss strategy.

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Poster

453. Monoamines and Other Regulators

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Topic: E.07. Food Intake and Energy Balance

Title: Blocking nitric oxide produces diet-dependent excitation or inhibition of feeding in rats with low motivation to eat

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[3]Psychology Dept. and Gonda Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel

Abstract: We tested the hypothesis that the unconventional neurotransmitter nitric oxide (NO) is an inhibitor of feeding when animals are in conditions of relatively low drive to eat, and therefore eat snacks, rather than eating large meals. The hypothesis is based on data from the marine slug *Aplysia* that both the amino acid L-arginine (the precursor from which NO is synthesized), as well as NO inhibit feeding when animals are only weakly motivated to eat. We reasoned that inhibitory feedback control of feeding by a nutrient such as L-arginine, via its effects on NO synthesis, might be a generally useful mechanism in many animals. To establish conditions of low drive to eat, adult male Wistar rats were provided during the night with 60% of the chow that they regularly consume and then in the morning, they were presented with abundant (20 g) chow for 0.5 hrs. After they had eaten, self-groomed and settled down, they received an intra-peritoneal injection of either N (G)-nitro-L- arginine methyl ester (L-NAME - 50 mg/kg)), a competitive inhibitor of L-arginine for access to NO synthase (NOS), or saline. They were then offered an additional 20 g of chow for 1.0 hrs, and feeding during this second period of food access was measured. L-NAME significantly increased measures related to feeding behavior: time spent in the vicinity of food (regular chow) - either sniffing or eating it, number of meals, and grams eaten. In a second experiment we used the same method but for the second morning feeding we replaced regular chow with a highly attractive high fat food. Interestingly, L-NAME caused an opposite effect, significantly reducing food intake compared to saline-treated control rats. This result is consistent with findings of others that NO mediates orexic effects of ghrelin and PYY. The findings support the hypothesis that NO is a weak inhibitor of feeding in mammals, and its inhibitory effects are prominent in conditions of low feeding motivation, but it is a facilitator of feeding when eating motivation is increased by highly palatable food.

Disclosures: **N. Hazut:** None. **A. Susswein:** None. **A. Weller:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: CONACyT Grant 129103 to OPG

DGAPA-UNAM Grant IN224314 to OPG

Title: Synthesis and characterization of a novel cannabinoid receptor 1 antagonist, ENP 11

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[1]Fisiologia, [2]Farmacia, [3]Psicofisiología, UNAM, Mexico, DF, Mexico

Abstract: The incidence of obesity remains a health problem worldwide. Pharmacologic antagonism of cannabinoid 1 receptor (CB1R) suppresses food ingestion, promotes weight loss, and improves metabolic profile in animals and humans. A newly synthesized analog of SR141716A, the 1-(2,4-Difluorophenyl)-4-methyl-N-(1-piperidinyl)-5-[4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carboxamide (ENP 11), has exhibited some properties as CB1R antagonist that are potentially useful to reduce food ingestion. Intra-peritoneal administration of 0.5, 1.0, and 3.0 mg/kg of ENP 11 were used to test its effects on Wistar rats (250 -300 g) food ingestion. Additionally, we evaluated core temperature, pain perception, and motor control. Results showed that ENP 11 doses presently tested reduced food ingestion during the first hour immediately after administration. Likewise, one of the used doses (1.0 mg/kg) of ENP 11 was able to blocking anandamide-induced hyperphagia during the first 4 hours of the dark cycle. An ENP 11 lower dose (1 mg /kg) blocked anandamide-induced hypothermia. However, none of the ENP 11 used doses affects pain perception or motor control. We concluded that ENP 11 is potentially useful to control food ingestion.

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Poster

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Support: CONCYTEG Grant 07-16-k662-054

CONACYT Grant 162016

Title: High-fat diet decrease GABA concentration in the frontal cortex of rats

Authors: *C. SANDOVAL SALAZAR, J. RAMIREZ-EMILIANO, S. A. TREJO-BAHENA, M. SOLÍS-ORTIZ
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Abstract: The inhibitor neurotransmitter γ-aminobutíric acid (GABA), the dopamine and the frontal cortex have a role in food intake. Some studies reported that chronic intake of a high-fat diet produce changes in GABA and dopamine concentration, in brain áreas involved in reward circuit and cognition. However the effect of a high-fat diet on frontal cortex is not well known. The aim of this study was to explore the effect of a high-fat diet on GABA and dopamine levels in the frontal cortex of rats. A total of 14 healthy adult male Wistar rats were analized in the present study. Seven rats were fed with standard diet and seven rats were fed with high-fat diet during eight weeks, respectively. Subsequently, the brain tissue of frontal cortex and a blood sample were obtained. The glucose, cholesterol and triglycerides were obtained using enzimatic methods. GABA level was determined by HPLC and dopamine by ELISA. The high-fat diet significantly increased the weight gain ($p=0.013$) and glucose levels ($p=0.005$) of rats compared to standar diet. The GABA concentration in the frontal cortex of rats with high-fat diet was significantly decreased ($p=0.04$) as compared with standard diet. The dopamine concentration did not show significant change in frontal cortex in both groups. This results indicate that exposure to hypercaloric diet decrease the GABA concentration in the prefrontal cortex, suggesting a disturbance in inhibitory process of food intake impacting in weight gain.

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant NS075820

Title: Methylation of a thiols and thioethers by human indolethylamine-n methyl transferase

Authors: *T. A. MAVLYUTOV[1,2], U. B. CHU[1], A. SCHULMAN[1], E. BAKER[1], R. RAJ[1], M. L. EPSTEIN[1], N. V. COZZI[3], L.-W. GUO[2], A. E. RUOHO[1]
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Abstract: Indolethylamine-N Methyl Transferase (INMT), a member of a family of small molecule methyltransferase enzymes, adds methyl groups from S-Adenosyl-L-Methionine (SAM) to the side chain amino group of indole amines such as tryptamine and serotonin; for example, tryptamine methylation produces N-monomethyl tryptamine (MMT) and the hallucinogen, N,N dimethyltryptamine (DMT). In animals, the endogenous production of DMT has been proposed to be an important regulator of mood and cognition through activation of serotonin and other receptors. In contrast to the INMT from human, rabbit, rat and chicken, INMT from mouse (referred to as ThioEtherMethylTransferase - TEMT) has been independently characterized as an enzyme that utilizes SAM to methylate sulfur, selenium and tellurium compounds to produce their respective "onium " products (Mozier et al. JBC 263,10 4527-4531 (1988)). TEMT may thus regulate the levels of endogenous thiols and trace metal containing compounds. To explore the functional differences and similarities between human INMT (hINMT) and mouse TEMT (mTEMT), we evaluated the efficiency of TEMT to produce MMT and DMT using the co substrate 14C-SAM. TEMT in mouse lung homogenates produced negligible amounts of N-methylated tryptamine derivatives when compared to rabbit lung homogenates. Similarly pure recombinant mTEMT was not efficient in tryptamine methylation compared to the methylation of thiol containing compounds such as cysteamine and methyl thio ethyl amine (MTEA). The Km for methylation of MTEA by TEMT was found to be 0.7 mM. On the other hand, pure recombinant hINMT methylated both cysteamine, N-acetyl cysteamine and MTEA as well as tryptamine. The Km for MTEA methylation at pH 7.2 was determined to be approximately 1.17mM compared to a previously reported tryptamine Km of 2.9 mM (Thompson et al. Genomics 61, 285-297 (1999)). Furthermore, over the pH range of 6.3-8.0, we found that more acidic conditions favored methylation of MTEA, while alkaline pH favored methylation of tryptamine. At pH 6.8, which is the average intracellular pH, the methylation levels of 10 mM tryptamine and 10 mM MTEA were approximately equal. These data suggest that hINMT regulates the metabolism of thiols and possibly similar selenium and tellurium derivatives and may thus compete with tryptamine (and serotonin) methylation especially under conditions of intracellular pH fluctuations (e.g. increased acidification).

Disclosures: T.A. Mavlyutov: None. **U.B. Chu:** None. **A. Schulman:** None. **E. Baker:** None. **R. Raj:** None. **M.L. Epstein:** None. **N.V. Cozzi:** None. **A.E. Ruoho:** None. **L. Guo:** None.

Poster

453. Monoamines and Other Regulators

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Program#/Poster#: 453.12/QQ20

Topic: E.06. Thirst and Water Balance

Title: Galanin like peptide gene expression in neural lobe of rat pituitary; effect of milk deprivation and refeeding

Authors: M. GOTO, *Y. YAMAMOTO, K. KUBO, M. ISHII, R. SAITO, S. ARAKI, R. KAWAGOE, Y. KAWADA, K. KUSUHARA
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Abstract: Galanin-like peptide (GALP), which is restrictively expressed in the hypothalamic arcuate nucleus (ARC) and pituicytes in the posterior pituitary gland (PP), is thought to be involved in the regulation of food intake, energy metabolism and reproduction. Recent studies in adult animals demonstrated that osmotic stimulation by dehydration and salt loading induce an increase in the GALP mRNA in the PP. Thus, it is suggested that GALP in the PP is implicated in the regulation of plasma osmolality and neurohypophysial hormones. However, the physiological role of GALP in the PP during postnatal development is unknown. We examined the effect of milk deprivation and refeeding on the GALP gene expression in the PP during preweaning period, using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Milk deprivation is commonly used as water deprivation in the preweaning period. After 24 h milk deprivation, the GALP gene expression was significantly increased compared with control animals both postnatal day 10 and 17. Forty-eight h milk deprivation caused significant increase in the GALP gene expression compared with control in postnatal day 10 and 17, and compared with 24 h deprived animals in postnatal day 17. Next, 24 h refeeding after 24 h milk deprivation induced significant decrease of the GALP gene expression compared with 24 h and 48 h deprived animals both postnatal day 10 and 17. Significant increase after milk deprivation and decrease after milk refeeding in the expression of the GALP gene suggested that osmolality change induced by milk deprivation and refeeding might be a stimulant to regulate the expression of the GALP gene in the preweaning period. GALP might have roles in the control of water drinking and osmolality regulation in the preweaning period.

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Poster

453. Monoamines and Other Regulators

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Topic: E.02. Neuroimmunology

Title: Interleukin-1 receptor-expressing cells in the arcuate hypothalamus mediate peripheral interleukin-1-induced hypophagia

Authors: *J. KONSMAN[1], L. CHASKIEL[2], A. BRISTOW[3], R. DANTZER[4]
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Abstract: Although the reduction in food intake observed in acute infectious and inflammatory diseases has been proposed to represent a regulated adaptive response, the underlying mechanisms remain incompletely understood. Our previous work has shown that the pro-inflammatory cytokine interleukin-1 (IL-1) can act in the brain to alter behavior during peripheral inflammation. The arcuate nucleus of the rat hypothalamus plays a pivotal role in the regulation of food intake and expresses the signaling interleukin-1 receptor (IL-1R1) (Ericsson et al., J. Comp. Neurol., 1995). However, lesioning of the neuropeptide Y(NPY)- and proopiomelancortin(POMC)-expressing neurons, the two major neuronal populations in the arcuate nucleus regulating food intake, does not attenuate the reduction of food intake after peripheral interleukin-1 administration (Reyes & Sawchenko, J. Neurosci., 2002). Besides neurons, venules and glia constitute the main nervous cell types expressing the signaling interleukin-1 receptor. Moreover, glial cells, and in particular tanycytes in the arcuate nucleus, have been proposed to play a role in the regulation of food intake (Bolborea & Dale, Trends Neurosci., 2013). In the present work, we set out) to determine if IL1-R1-expressing cells in the hypothalamus mediate reduced food intake in response to peripheral IL-1 administration, and 2) if so, to identify the cell types involved. Cells expressing IL-1R1 were killed by infusion of IL-1 coupled to the intracellular toxin saporin (IL-1-SAP) into the arcuate hypothalamus. Control infusions consisted of uncoupled IL-1 and saporin and PBS. At least one week later rats were injected intraperitoneally with IL1. Intra-arcuate IL-1-SAP attenuated the reduction in food intake after peripheral administration of IL-1, indicating that arcuate cells mediate IL-1-induced hypophagia. *Post mortem* histochemical analyses of brain sections of the same animals revealed that intra-arcuate IL-1-SAP reduced the number of NPY-neurons, without affecting the number of POMC-neurons or the surface covered by tanycytes. Taken together, these findings indicate that IL-1R-bearing NPY neurons in the arcuate nucleus take part in the reduction of food intake after peripheral IL-1 administration and suggest that hypophagia observed in infectious and inflammatory diseases reflects, at least in part, a regulated response.

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Poster

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Title: Glucagon-like peptide-1 (GLP-1) modulation of shaker potassium channel (Kv1.3) in the olfactory bulb

Authors: *N. THIEBAUD[1], I. LLEWELLYN-SMITH[3], F. GRIBBLE[4], F. REIMANN[4], S. TRAPP[5], D. A. FADOOL[2]
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Abstract: Olfactory sensitivity has long been known to correlate with appetite. Nutrients and hormones have the capacity to modulate the activity of neurons involved in the transmission of olfactory information, particularly mitral cells (MC) of the olfactory bulb (OB), suggesting an intimate link between the endocrine system and olfactory processing. Previous studies performed by our group have shown that the voltage-gated potassium channel, Kv1.3, can be a downstream target for MC modulation by metabolic cues such as insulin or glucose. Here, we investigated the role of glucagon-like peptide-1 (GLP-1), an incretin peptide that has several roles, including the suppression of food intake, increase in heart rate, and neuroprotection. Using transgenic mice expressing YFP under the control of the preproglucagon promoter, we have identified a population of GLP-1-producing neurons in the granule cell layer, as well as a strong immunoreactivity for the GLP-1 receptor in the MCs, suggesting a paracrine effect of GLP-1 in the OB. Whole-cell patch-clamp recordings in acute OB slices showed that bath perfusion of GLP-1 or its stable analogue, exendin-4, resulted in a significant increase in the evoked action potential frequency and a concomitant decrease in the interburst interval in MCs. Interestingly, such modulation was absent in OB slices from mice with targeted deletion of Kv1.3 (Kv1.3-/-).

In voltage-clamp experiments, there is a reduced K conductance in MCs following the application of GLP-1 in slices from WT but not Kv1.3-/- mice. These results demonstrate that Kv1.3 is important for GLP-1 signaling in the OB, making both the receptor and the channel relevant activity-dependent targets to modulate olfaction.

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Poster

453. Monoamines and Other Regulators

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Program#/Poster#: 453.15/QQ23

Topic: E.03. Behavioral Neuroendocrinology

Support: Fapesp

Title: Effects of chronicle central insulin infusion on food intake in female rats in different reproductive states

Authors: ***A. C. KISS**[1], A. A. NUNES[1], M. O. KLEIN[2,3], L. F. FELÍCIO[2], B. WOODSIDE[4]
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Abstract: High levels of insulin act on the brain to decrease food intake. Research in animals showed that intracerebroventricular (ICV) administration of insulin decreases food intake and body weight. However most of these studies were conducted on males and it is well established that food intake in females varies according to the stage of the estrous cycle and their reproductive state. Further, acute ICV injection of insulin is more effective in reducing food intake in male rats than in females and this difference has been attributed to central effects of estrogen. These sex differences have been observed in studies using acute rather than chronic central insulin infusion. The present study, therefore, investigated the effects of chronic central insulin infusion on food intake in female rats in different reproductive states. Female Wistar rats were randomly assigned to one of 6 experimental groups: intact saline (IS, n=6), intact insulin (II, n=6), ovariectomized saline (OS, n=10), ovariectomized insulin (OI, n=10), lactating saline (LS, n=10), and lactating insulin (LI, n=10). Rats from OS and OI groups were ovariectomized

on postnatal day (PND) 75, 15 days before cannula placement surgery. Rats from LS and LI groups were mated around PND 75 and delivered naturally. All rats underwent surgery for cannula placement in the lateral ventricle (PND 90 for intact and ovariectomized rats, and one day after parturition for lactating rats). Osmotic pumps delivered either saline or 10mU of insulin per day at a rate of 0.5µl/hour for 7 consecutive days. Body weight and food intake were recorded daily. Rats were submitted to glucose tolerance test. There were no significant effects of ICV insulin infusion on food intake, body weight or glucose tolerance in any of the reproductive states examined.

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Poster

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DGAPA-UNAM IN206712

Title: Fasting enhances pyroglutamyl peptidase II activity in tanycytes of the mediobasal hypothalamus and thyroliberinase in the serum of male adult rats

Authors: I. LAZCANO[1], P. JOSEPH-BRAVO[1], E. SÁNCHEZ[2], *J.-L. CHARLI[3]
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Abstract: Fasting decreases activity of the hypothalamus-pituitary-thyroid (HPT) axis and preserves energy reserves. The parvocelular paraventricular nucleus of the hypothalamus (PVN) neurons that secrete thyrotropin releasing hormone (TRH) into the portal capillaries regulate thyrotropin secretion in mammals. Synthesis and secretion of TRH is reduced during fasting. Pyroglutamyl peptidase II (PPII), the thyrotropin releasing hormone (TRH)-degrading ectoenzyme, is expressed in tanycytes of the median eminence (ME), and may be localized near

the portal capillary loops from where it may control the amount of TRH that reaches the anterior pituitary. Except for the stimulatory role of thyroid hormones on expression and activity of PPII in the ME, the physiological contexts that regulate tanycyte PPII are unknown. We tested the hypothesis that tanycyte PPII activity is regulated during fasting. PPII mRNA expression and activity were measured in the median eminence of male Wistar rats submitted to fasting for 36-72 h. We also measured the activity of thyroliberinase, the serum isoform of PPII secreted by the liver, and PPII activity in anterior pituitary. Fasting reduced animal weight, PVN TRH mRNA levels, serum TSH, and thyroid hormones (total T3 and T4) concentrations. Semi-quantitative *in situ* hybridization (ISH) indicated that PPII mRNA levels increased in tanycytes 48 h after fasting initiation. This increase was transitory (not detected at 72 h) and concomitant with, or followed by, an increase of PPII activity in the median eminence. An increase in D2 mRNA levels in the median eminence was also detected by ISH. The subcutaneous infusion of leptin did not reverse the increase of PPII activity in male rat median eminence. Thyroliberinase activity was increased at most time points, whereas adenohypophysis PPII activity was not affected by fasting. We conclude that during fasting increases in median eminence PPII and serum thyroliberinase activities may contribute to the maintenance of a profound reduction of HPT axis activity.

Disclosures: I. Lazcano: None. **P. Joseph-Bravo:** None. **E. Sánchez:** None. **J. Charli:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.17/QQ25

Topic: E.01. Neuroendocrine Processes

Support: The New Zealand Marsden Fund

Title: Is unopposed ghrelin signaling a cause of infertility and obesity in leptin receptor deficient mice?

Authors: *C. ANCEL, S. A. GEORGE, M. INGLIS, G. M. ANDERSON
Dept. of Anat., Ctr. For Neuroendocrinology, Dunedin, New Zealand

Abstract: The hormone leptin suppresses appetite and is required for fertility. Mutations in the leptin receptor gene cause obesity and infertility. Ghrelin is a hormone that stimulates appetite and can suppress gonadotrophin secretion. An antagonistic relationship between leptin and

ghrelin has been suggested. The aim of this study was to examine whether infertility in leptin receptor-deficient mice results from the absence of an opposing force to ghrelin's suppressive influence. Puberty onset and estrous cycles were analyzed in four groups of female mice: ghrelin knockout (ghrelin -/-), leptin receptor knockout (db/db), ghrelin and leptin receptor knockout (double knockout) and wild-type littermate controls (WT). The ghrelin -/- mice underwent normal vaginal opening, comparable to that of the WT littermates, whereas the db/db and double knockout mice had delayed (by 7 d) vaginal opening. Vaginal cytology provided evidence of normal cycles in the WT and ghrelin -/- mice, but not in the db/db or double knockout mice. As expected, the db/db mice were ~70% heavier than both the WT littermates and the ghrelin -/- mice by 6 weeks of age ($P<0.001$). Interestingly, the double knockout mice were 10% lighter than the db/db mice ($P<0.05$), although they remained significantly heavier than both the WT controls and single ghrelin -/- mice. These data support the idea that unopposed ghrelin actions may be a partial cause of body weight gain in db/db mice, but this does not appear to be a significant factor behind their infertility.

Disclosures: C. Ancel: None. **S.A. George:** None. **M. Inglis:** None. **G.M. Anderson:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.18/QQ26

Topic: E.07. Food Intake and Energy Balance

Support: NIH R37 DK35254

Title: Role of peroxisome proliferator-activated receptor γ in appetite control

Authors: *J. GARRETSON, V. RYU, B. TEUBNER, T. BARTNESS
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Abstract: Background: Rosiglitazone (Rosi), a clinically used peroxisome proliferator-activated receptor gamma (PPARγ) agonist, is used in type II diabetes treatment; however, increases in food intake and body mass/fat are common side-effects. The underlying mechanisms for these effects are incompletely known as is the endogenous role of PPARγ in appetite control. Therefore, we tested the role of stimulating PPARγ in Siberian hamsters, a model of human appetite. Methods: We examined effects of third ventricular (3V) Rosi on Siberian hamster food hoarding, then tested whether food deprivation (FD) increases agouti-related protein (AgRP) and

PPARγ mRNA in Siberian hamsters and C56BL/6 mice. We further tested whether ip Rosi increases both PPARγ and AgRP mRNA in ad libitum-fed hamsters and mice as well as if a PPARγ antagonist (GW9662) would block FD-induced increased AgRP+PPARγ mRNA. Results: 3V Rosi increased food hoarding cumulatively [lasting for 7d, a persisting effect similar to 3V AgRP and FD]. FD (hamsters 48h; mice 24h) increased AgRP mRNA expression within the arcuate nucleus (ARC) in both species with concomitant increases in PPARγ mRNA almost exclusively within ARC/median eminence AgRP neurons. Rosi increased AgRP mRNA expression similar to FD and GW9662 blocked FD-induced increases in AgRP mRNA expression in both species. Conclusions: We demonstrated for the first time that PPARγ is sufficient to increase AgRP mRNA expression, as well as necessary for FD-induced increases in AgRP mRNA expression. These findings provide initial evidence that FD-induced increases in AgRP may be a PPARγ dependent process that also affects ingestive behaviors.

Disclosures: J. Garretson: None. **V. Ryu:** None. **B. Teubner:** None. **T. Bartness:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.19/QQ27

Topic: E.07. Food Intake and Energy Balance

Support: Grant-in-Aid for Exploratory Research (No.25670358) from Japan Society for the Promotion of Science (JSPS)

Title: Klotho is a fasting-induced gene in the hypothalamus

Authors: *T. KOMORI, Y. MORIKAWA
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Abstract: Hypothalamus is well-known to be important for the regulation of energy homeostasis including the control of food intake and energy expenditure. Previously, we have reported that fasting induces the activation of extracellular signal-regulated kinase and cAMP response element binding protein in the hypothalamus (Morikawa Y, et al. J Neuroendocrinol, 2004), suggesting that the expression of some genes is induced in the hypothalamus in response to fasting. Using cDNA microarray analysis, we found some genes induced by fasting in the hypothalamus, one of which was klotho. Consistent with the data in cDNA microarray analysis, the expression of klotho protein was induced by fasting in the hypothalamus, especially in the arcuate nucleus. There are two types of neurons in the arcuate nucleus of the hypothalamus: the pro-feeding (anabolic) neuropeptide Y (NPY)/agouti-related protein (AgRP)-expressing neurons

and the feeding-inhibitory (catabolic) proopiomelanocortin (POMC)-expressing neurons. The expression of klotho was observed in NPY/AgRP neurons, but not in POMC neurons. It has been reported that klotho makes the receptor complex with a fibroblast growth factor receptor (FGFR). Double-immunofluorescence staining revealed that klotho was colocalized with FGFR1 and FGFR2 in the arcuate nucleus during fasting. In addition, the expression of FGF23, a ligand for klotho/FGFR complex, was induced by fasting in the hypothalamus. These results suggest that klotho is involved in some important functions of NPY/AgRP neurons, such as the regulation of food intake, in the fasted state. To gain insights into the function of klotho in the hypothalamus during fasting, we analyzed heterozygous klotho mutant (kl/+) mice because homozygous klotho-mutant (kl/kl) mice have a short life span and show severe physical abnormalities consistent with premature aging. Western blot analysis revealed that the protein expression of klotho in the hypothalamus was reduced more than 30% in kl/+ mice compared to wild-type (WT) mice in the fed states. Fasting induced the expression of klotho in the hypothalamus of WT mice, but not in kl/+ mice. There was no significant change in the expression of FGF23 between WT and kl/+ mice in both fed and fasted states. The body weights in kl/+ mice were similar to those in WT mice in both fed and fasted states. In addition, there were no changes in the expression of NPY and AgRP between WT and kl/+ mice. From these findings, klotho may not be related to the expression of these neuropeptides, but might involved in the other function of NPY/AgRP neurons, including the excitation of neurons and the expression of other genes.

Disclosures: **T. Komori:** None. **Y. Morikawa:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

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Program#/Poster#: 453.20/QQ28

Topic: E.07. Food Intake and Energy Balance

Support: MRC grant G0902250

The Royal Society Research Grant

Title: A role for calorie restriction and ghrelin on immediate early gene expression and hippocampal plasticity: Implications for learning and memory

Authors: *J. S. DAVIES[1], A. K. E. HORNSBY[1], Y. T. REDHEAD[1], T. WELLS[3], Z. B. ANDREWS[4], M. R. BROWN[2]
[1]Col. of Med., [2]Col. of Engin., Swansea Univ., Swansea, United Kingdom; [3]Cardiff Univ., Cardiff, United Kingdom; [4]Monash Univ., Melbourne, Australia

Abstract: It is well established that there is an important relationship between nutritional status and cognitive function, with calorie restriction (CR) enhancing performance in memory tasks in rodents (Halagappa et al.2007) and in elderly humans (Witte et al.2009). The mechanisms underlying this relationship are not well understood. However, CR is known to increase levels of BDNF and neurogenesis in the hippocampal dentate gyrus (DG) (Lee et al.2002). To date, the only factor directly elevated by CR is the hormone, ghrelin. Ghrelin released from the stomach during CR (Kojima et al.1999) crosses the BBB and binds to its receptor in the hippocampus to promote memory (Diano et al.2006). As ghrelin is not produced in the brain (Sakata et al.2009), the responsiveness of ghrelin-sensitive neurons to circulating levels represents a mechanism connecting nutritional status with neuronal function and potentially with neurogenesis and cognition. In this study, we have investigated the role of the orexigenic stomach hormone, ghrelin, along with its receptor, Ghsr, in mediating the beneficial effects of CR on hippocampal plasticity. First, we used the Ghsr-GFP mouse to confirm expression of Ghsr in extra-hypothalamic sites, including the DG, entorhinal cortex (EC), and cingulate cortex (CC). Next, to determine that the beneficial effects of ghrelin on hippocampal function are mediated via Ghsr we treated Ghsr-null mice and WT littermates with ghrelin by osmotic mini-pump (7-day i.v 80ug/day). Subsequent analysis showed that ghrelin enhanced DG cell proliferation ($Ki67^+$, $P<0.05$) in a Ghsr-dependent manner. Furthermore, genome-wide expression profiling (Illumina Mouse Ref8 BeadChip) of hippocampi from these mice identified the plasticity related genes, Fos, NeuroD2, BMP1, as ghrelin-Ghsr regulated transcripts (>1.5 fold-change, $P<0.05$). Finally, in Ghsr-GFP mice, we raised ghrelin levels directly via injection (10ug/kg i.p), indirectly via CR (overnight fast), or with injection and CR. 16h after directly elevating ghrelin, expression of the immediate early gene, Egr-1, was increased in the DG, CA1, EC ($P<0.01$) and CC ($P<0.05$). CR elevated DG Egr-1 expression ($P<0.05$), whilst the combination of ghrelin and CR also increased Egr-1 expression in CC Ghsr-GFP^+ neurons ($P<0.01$). Together, these studies provide evidence for the role of CR and ghrelin in modulating higher brain centers implicated in memory.

Disclosures: J.S. Davies: None. **A.K.E. Hornsby:** None. **Y.T. Redhead:** None. **T. Wells:** None. **Z.B. Andrews:** None. **M.R. Brown:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

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Program#/Poster#: 453.21/QQ29

Topic: E.07. Food Intake and Energy Balance

Support: Natural Sciences and Engineering Research Council

Canadian Institutes of Health Research

Ontario Graduate Scholarship

Canada Foundation for Innovation

Title: The interaction of ghrelin and endocannabinoid systems within the VTA is necessary and important for the modulation of food intake

Authors: *A. W. EDWARDS, S. ROSENBAUM, A. ABIZAID
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Abstract: Ghrelin is a hormone that targets growth hormone secretagogue receptors (GHSR) in the brain to increase food intake and energy balance. Its ability to stimulate food intake is well known in the hypothalamus (HYP), a brain region integral to the modulation of feeding; however, evidence suggests that ghrelin also increases appetite by acting on GHSRs in the ventral tegmental area (VTA), a brain region associated with reward seeking behaviors. Interestingly, it has been shown that endogenous cannabinoids (i.e. endocannabinoids), like ghrelin, stimulate appetite within the HYP and that a functional endocannabinoid system (ECS) is required for ghrelin's appetitive effects within this region. We hypothesize that a similar interaction between ghrelin and endocannabinoid systems exists in the VTA to modulate feeding. We predicted that if this interaction exists and is important in modulating feeding then there should be differences in cannabinoid receptor (i.e. CB-1R) expression between WT and rats which lack functional GHSR (i.e. GHSR KO), especially within important feeding nuclei (e.g. prefrontal cortex (PFC), hippocampus (HIP), nucleus accumbens (NA), HYP and VTA). To investigate this, these brain regions from Fawn hooded GHSR KO and WT rats were punched, processed, and analyzed *via* Real Time qPCR using the $2^{-\Delta\Delta Ct}$ method, to compare the relative expression of CB-1R mRNA. Although no significant differences in CB-1R expression were found within HYP, HIP, and NA regions ($p > .05$), GHSR KO rats had significantly lower VTA CB-1R mRNA expression but higher PFC CB-1R mRNA expression than WT rats ($p < .05$). Given that disrupting ghrelin signalling induced changes in CB-1R expression within the VTA we wanted to test whether a functional ECS was important for modulating ghrelin's orexigenic effects within the VTA. To test this hypothesis, Long-Evans rats were cannulated and placed in one of the following 4 treatment groups (intraperitoneal/intra-VTA): vehicle/saline, rimonabant (1.5 mg/kg)/saline, vehicle/ghrelin (1 µg /0.5 µl), rimonabant (1.5 mg/kg)/ghrelin (1 µg /0.5 µl) to determine if global pharmacological inhibition of the cannabinoid system would attenuate the

ability of ghrelin within the VTA to acutely increase food intake (measured 1, 2, 4, & 6 hours post-microinjection). Results demonstrated that ghrelin administered into the VTA significantly increased food intake ($p < .05$) and that this effect was attenuated to control levels when animals were pre-treated with rimonabant 30 minutes prior to ghrelin microinjections. This data ultimately suggests that ghrelin targets the VTA to increase food intake through an interaction with the CB system.

Disclosures: A.W. Edwards: None. **S. Rosenbaum:** None. **A. Abizaid:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.22/QQ30

Topic: E.07. Food Intake and Energy Balance

Title: A novel effect for xenopsin: stimulating food intake

Authors: *B. MCCONN, J. PARK, E. R. GILBERT, M. A. CLINE
Virginia Tech., Blacksburg, VA

Abstract: Xenopsin, an 8 amino acid peptide, was first isolated from amphibian skin and later found in mammalian and avian gastrointestinal systems. Because xenopsin is structurally related to xenin and neurotensin, which both affect food intake, we hypothesized that xenopsin would also affect feeding. We performed intracerebroventricular (ICV) injections of 0, 0.3, 1.0, and 3.0 nmol xenospin to ad libitum fed 5 day post-hatch Hubbard x Cobb-500 chicks and found that 1.0 and 3.0 nmol doses increased food intake. On a cumulative basis this effect persisted for 150 min following injection, while none of the doses affected water intake. Following this, we ICV administered 3.0 nmol xenposin and measured c-Fos immunoreactivity in key hypothalamic nuclei associated with appetite: the lateral hypothalamic area, ventromedial hypothalamus, dorsomedial hypothalamus, arcuate nucleus and the magno- and parvo-cellular divisions of the paraventricular nucleus. Only the lateral hypothalamus had increased c-Fos immunoreactivity. A comprehensive behavior analysis was conducted and chicks that received 3.0 nmol ICV xenopsin had decreased time sitting and increased feeding pecks. Time spent preening, perching, and sitting was not affected and the number of vocalizations, jumps, steps, exploratory pecks, drinks, and escape attempts were not affected. These results demonstrate that ICV xenopsin injection causes increased food intake involving the lateral hypothalamic area, and does not affect a wide range of other behaviors.

Disclosures: **B. McConn:** None. **J. Park:** None. **E.R. Gilbert:** None. **M.A. Cline:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.23/QQ31

Topic: E.07. Food Intake and Energy Balance

Support: NINDS Intramural Program

Title: A neuropeptide initiates feeding pauses in Trichoplax adhaerens

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Abstract: Trichoplax adhaerens is an early-diverging metazoan that feeds by external digestion of microalgae and cyanobacteria on marine surfaces on which it crawls by ciliary gliding. It pauses periodically and the frequency of pauses is related to the concentration of food (Ueda, Koya, & Maruyama, 1999). Despite its coordinated behaviors it appears to lack both electrical and chemical synapses, raising the question how these behaviors are initiated and controlled. Timelapse transmitted light and fluorescence imaging of Trichoplax feeding on the red microalgae showed that pauses are associated with feeding, evident by the release of fluorescent phycobiliproteins from underlying algae when digestion begins. Groups of Trichoplax, including individuals not in direct contact, frequently pause simultaneously, suggesting that a signal is passed between animals through the medium. Gland cells arrayed around the periphery of Trichoplax contain an FMRFamide-like neuropeptide in their secretory granules, identified by immunofluorescence, and there is genomic evidence for the presence of additional neuropeptides. The possibility that a neuropeptide initiates the feeding pauses in Trichoplax was explored by applying synthetic peptides to crawling animals. Pauses, but no other feeding behavior, occurred reproducibly within < two minutes after bath application of 30μM FMRFamide, or a single pulse of 5 μM Trichoplax DGQFFNP-amide from a pipette positioned less than one mm away. Visualization of cilia by DIC microscopy showed that all the cilia in an individual are arrested during a spontaneous or induced pause. Thus, Trichoplax, an animal reported to lack synapses, may secrete a neuropeptide to signal ciliary arrest and stop locomotion during external digestion of algae. We are searching for additional non-synaptic signaling systems in Trichoplax controlling other steps in the complex feeding behavior of Trichoplax.

Disclosures: **C. Smith:** None. **T.S. Reese:** None. **E. Hamid:** None.

Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.24/QQ32

Topic: E.03. Behavioral Neuroendocrinology

Support: FAPESP grant 2012/07378-6

Title: Maternal high fat nutrition and its transgenerational effects

Authors: ***M. O. KLEIN**[1,2], C. N. TOBARUELA[1], E. TEODOROV[1,3], A. C. I. KISS[1,4], L. F. FELICIO[1]
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Abstract: A bad nutrition during pregnancy and lactation may cause metabolic and behavioral deleterious effects to the mother and her descendents. Additionally, adequate maternal care is important for offspring development. Therefore, the present study aimed to evaluate the impact of maternal high fat nutrition in F0 and F1 generations on maternal behavior, body weight gain during pregnancy and lactation and glucose levels during pregnancy. For F0 generation, pregnant female Wistar rats were assigned either to: high fat group (HF, 45% fat diet from day 0 of pregnancy to day 21 of lactation; n=10) or control group (standard diet - 4% fat, during the same period; n=9). After weaning, female offspring were further divided, thus half of the litter was kept with the same diet as the mother, and the other half changed the type of diet. It came up with 4 groups for F1 generation: CC (standard diet for all life; n=8); CHF (standard diet before weaning, HF after weaning; n=8); HFC (HF diet before weaning, control diet post-weaning; n=8); HFHF (HF diet for all life; n=10). On postnatal day (PND) 90, F1 females were mated. During their pregnancy and lactation, females continued with the same diet of post-weaning. Both generations had maternal behavior, body weight gain during pregnancy and lactation, and glucose levels during pregnancy evaluated. Maternal behavior was analyzed on 5th and 10th days of lactation. On the test day, all pups were removed from the home cage and the nest was destroyed. After 30 minutes, the pups were returned to the cage and mother-pup interaction was

recorded for 30 min. Pup retrieval latencies, pup grooming, self grooming, total time of crouching, total time off pups, and nest building were observed. For both generations, females spent more time crouching on pups on day 10. F1 dams spent more time grooming their pups and retrieved them more times on day 5, and spent more time off pups on day 10. There were no differences among groups. As expected, there was a significant effect of time on body weight gain during pregnancy and lactation, but during lactation HF dams gained less weight than control ones. CHF and HFHF groups also gained less weight during pregnancy and lactation. As expected, there was a significant effect of time on glucose levels but there were no significant differences among groups. In conclusion, maternal high fat nutrition did not impair overall display of maternal behavior, but reduced the body weight gain during pregnancy and lactation. Furthermore, there were no differences among the high fat diet effects between generations.

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Poster

453. Monoamines and Other Regulators

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 453.25/QQ33

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Neuropeptide Y regulates the hematopoietic stem cell niche in bone marrow

Authors: *J.-S. BAE[1,3], M. PARK[1,3], J. LEE[1,3], H. JIN[2]
[1]Dept. of Physiol., [2]Kyungpook Natl. Univ., Daegu, Korea, Republic of; [3]BK21 Plus KNU Biomed. Convergence Program, Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Many reports have shown that the sympathetic nervous system (SNS) is important for control of the bone marrow environment, although the role of neuropeptide Y (NPY) in the regulation of the bone marrow niche has not been systematically studied. Here we demonstrate that NPY deficient mice showed significantly reduced hematopoietic stem cell (HSC) numbers and impaired regeneration in bone marrow (BM) due to destruction of SNS fibers. NPY elevation led to HSC egress from BM into peripheral blood. Conditional knockout mice lacking the Y1 receptor in osteoblasts did not induce HSC mobilization after NPY injection. Further, the induced mobilization of HSCs by NPY relieved bone loss in ovariectomized mice. NPY also prevented bone marrow impairments from chemotherapy-induced SNS injury through Y1 receptors in macrophages. Therefore, these results suggest a new role of NPY as a regulator of the bone marrow niche, and highlight the potential therapeutic value of this neuropeptide. This

work was supported by the Bio & Medical Technology Development Program (2012M3A9C6049913) of the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning, Republic of Korea.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 454.01/QQ34

Topic: E.08. Biological Rhythms and Sleep

Title: The effects of larval ethanol exposure on type-2 photic phase shifting stimuli in period mutants of *Drosophila* melanogaster

Authors: *D. AMARAL, G. C. NASH, K. N. CARLSON, N. F. NASCIMENTO, D. PYNE, J. A. SEGGIO
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Abstract: The effects of light, drugs, and other stimuli on the circadian rhythm are identified through studies investigating the period of free-running rhythm or phase responses to light pulses. In such studies, altering the phase or period of the free-running rhythm is thought to reflect the underlying circadian pacemaker. Recent investigations have shown that larval-ethanol treatment can alter the period and period gene transcription of adult *Drosophila* period mutants, even after ethanol exposure has ceased. This study aims to uncover the effects of larval-ethanol exposure on the photic phase responses in period mutant fruit flies. *Drosophila* period mutant larvae were raised on food laced with either water or 10%-ethanol and upon eclosion were placed into activity monitors in LD for three days. On the last day of LD, light pulses at ZT 14 and ZT 21 were administered using an Aschoff Type-2 protocol. It was found that both perS and perL have increased phase delaying responses to light pulses at ZT 14 compared to wild-type CS. Additionally, while both perS and CS responded with normal sized phase advances to light pulses at ZT 21, perL showed shifts of approximately 10-hours, most likely due to their extremely long rhythm. Although ethanol causes changes in the period of the rhythm, it appears that larval-ethanol produces no changes in the responses to light pulses. These results indicate that developmental ethanol affects the period and phase differently in *Drosophila*, and that there are species differences in how ethanol affects the phase between flies and rodents.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

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Program#/Poster#: 454.02/QQ35

Topic: E.08. Biological Rhythms and Sleep

Support: NIH NS078220

Title: GABAA receptor δ and γ2 subunits are expressed in a 24 hour pattern in the suprachiasmatic nucleus of male syrian hamsters

Authors: ***J. C. WALTON**, H. E. ALBERS, J. K. MCNEILL, IV, A. P. ROSS
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Abstract: GABAA receptors (GABAARs) are pentameric assemblages generally comprised of three different proteins from 19 subunits. Although all GABAARs are ligand gated ion channels, subunit composition determines channel properties and location of the receptor on the cell membrane. Classical phasic inhibition is mediated by GABAARs containing the γ2 subunit located at synaptic sites, whereas tonically active GABAARs contain the δ subunit are high-affinity non-desensitizing channels found at extrasynaptic locations. Recent studies have revealed that expression levels of δ and γ2 subunits regulate the balance between tonic and phasic inhibition in multiple brain regions. In addition, GABAA δ receptors also have a specific role in the regulation of photic input into the SCN in a circadian phase-specific manner, however whether these receptor subtypes are differentially expressed over the LD cycle in the SCN is not known. Toward this end, we collected SCN tissues via micropunch from male Syrian hamsters exposed to a 14:10 L:D cycle at ZT1, 6, 13, 17, and 19 to assess GABAA δ and γ2 expression. In common with the circadian rhythm in the effects of GABAA δ receptors on photic input to the SCN, GABAA δ and γ2 mRNA expression varied across the LD cycle, with δ levels at nadir during the dark phase when γ2 expression levels were highest. We are currently assessing GABAA δ and γ2 protein across the LD cycle. Taken together, these findings indicate that there is a 24 hr rhythm in expression of the GABAA subunits that may contribute to the circadian pattern of responsiveness to GABA at extrasynaptic receptors in the SCN.

Disclosures: **J.C. Walton:** None. **H.E. Albers:** None. **J.K. McNeill:** None. **A.P. Ross:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

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Topic: E.08. Biological Rhythms and Sleep

Support: Project for Developing Innovation Systems of the MEXT

Title: Real time monitoring of clock gene expression in the suprachiasmatic nucleus from freely moving mice

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Abstract: In mammals, a master clock, the suprachiasmatic nucleus (SCN), entrains to environmental light dark cycles and transmits circadian signals to behaviors including sleep-wake cycles. According to a current hypothesis, the intracellular mechanism for rhythm generation is based on an autoregulatory transcription and translation feedback loop involving several clock genes and their protein products. A bioluminescence reporter, such as firefly luciferase, provides a powerful tool for long-term recording of clock functions. To understand circadian rhythms in a system level, it is important to assess gene expression in specific brain areas from conscious animals. In this research we successfully monitored clock gene expression rhythms in the SCN in freely moving mice and analyzed phase-responses of Per1 and Bmal1 expression rhythms to a single light exposure. We used Per1-luc and Bmal1-Eluc transgenic mice expressing a Per1 promoter- and Bmal1 promoter-driven luciferase reporters, respectively. We also used PER2::LUC knock-in mice carrying a PER2 fusion luciferase reporter. An optical fiber was stereotaxically set above the SCN through a guide cannula fixed on the skull. Luciferin was released at constant rate form an osmotic mini-pump implanted in the abdominal cavity. In some mice, luciferin was infused via lateral ventricle by connecting catheter to an osmotic mini-pump. The intensity of bioluminescence was monitored every minute by a photomultiplier tube connected to the optical fiber. Spontaneous movements were measured by an infrared thermal sensor. As a result, robust circadian rhythms in Per1-luc, Bmal1-Eluc, and PER2::LUC expression were detected in the SCN of freely moving mice continuously for more than 3 weeks. Comparing with *ex vivo* experiments, circadian phases of *in vivo* rhythms were slightly delayed but the phase relations among three genes were kept the same as *ex vivo*. In response to a 9h light

exposure from CT11.5, Per1-luc rhythms phase-delayed immediately, whereas Bmal1-Eluc rhythms gradually shifted with a transient period of 4-5 days. Importantly, the phase relations between the Per1-luc and the activity-onset, and between the Bmal1-Eluc and the activity-offset were kept constant. These results suggest differential roles of circadian Per1 and Bmal1 expression rhythms in the SCN in the regulation of behavior rhythms.

Disclosures: D. Ono: None. **K. Honma:** None. **S. Honma:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Title: Comprehensive transcriptomics of the suprachiasmatic nuclei uncovers LHX1 requirement for circadian behavior

Authors: *M. HATORI[1,2], S. GILL[2], L. MURE[2], M. GOULDING[2], D. D. M. O'LEARY[2], S. PANDA[2]
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Abstract: Tightly coupled suprachiasmatic nucleus (SCN) neurons sustain circadian rhythms and are selectively sensitive to changes in the ambient light:dark cycle. We used expression profiling to examine the transcriptional landscape of the mouse SCN. Hundreds of transcripts encoding basic cellular functions show circadian rhythms, most of which are not acutely affected by light. Light-induced transcriptional changes are gated by the circadian clock and mediated by retinal photoreceptors. Light pulse that phase shifts behavioral rhythm, induced transcripts that reset the clock and suppressed transcripts that mediate intercellular communication. LHX1 transcription factor expression is enriched in the SCN and suppressed by light. SCN-specific deletion of Lhx1 led to reduced expression of both the neuropeptide VIP and the receptor of AVP without affecting the overall SCN structure and the cell autonomous clock. LHX1 is a

central transcriptional regulator of neuronal communication that is critically required for the master oscillator function of the SCN.

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Poster

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Title: Distinct firing properties of vasoactive intestinal peptide- (VIP-) expressing neurons drive coordinated electrical activity in the suprachiasmatic nucleus

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Abstract: Previous studies have demonstrated that neurons in the suprachiasmatic nucleus (SCN) that express vasoactive intestinal polypeptide (VIP) play an important role in modulating rhythmicity and synchronizing neuronal firing. Anatomical studies suggest that as many as 10-20% of the neurons in the SCN express VIP but little is known about the firing properties of VIP neurons and how the activity of these cells regulates daily rhythms in the SCN. To begin to explore these questions, we have examined the diurnal passive and active membrane properties of VIP and non-VIP neurons in the mouse SCN. Whole-cell current clamp recordings were obtained from VIP and non-VIP neurons in acute SCN slices prepared from adult VIPcre-tdTomato knockin mice, which expresses the fluorescent tdTomato protein only in VIP cells, and firing rates, input resistances (R_{in}), resting membrane potentials (V_m), afterhyperpolarization (AHP) amplitudes and action potential durations at 50% repolarization (APD_{50}) were measured. These experiments revealed that both VIP and non-VIP neurons are spontaneously active with higher firing rates during the day. However, the mean ± SEM firing frequency determined in VIP neurons, both during the day (3.1 ± 0.2 Hz) and at night (2.2 ± 0.2 Hz) was significantly

($p<0.001$) higher than in non-VIP neurons. The mean ± SEM R_{in} measured, was decreased by ~40% in both VIP and non-VIP neurons at night ($p<0.05$). However, there were no significant differences in V_m between VIP and non-VIP neurons at any time of day. The membrane potential measured at the peak of the AHP was significantly ($p<0.001$) more hyperpolarized in VIP neurons, than in non-VIP neurons, during the day (-21.9 ± 0.8 mV) and at night (-24.6 ± 1.1 mV). In addition, action potentials are briefer in VIP, than in non-VIP neurons: the mean ± SEM APD_{50} measured during the day, for example, was significantly ($p<0.01$) shorter in VIP neurons (3.6 ± 0.1 ms) than in non-VIP neurons (4.4 ± 0.3 ms). Interestingly, the mean APD_{50} in VIP neurons was not significantly different at night. The briefer action potential durations and higher repetitive firing rates of VIP, compared with non-VIP, neurons during the day and night suggest that VIP neurons drive electrical activity in the SCN. In addition, the results here are consistent with the idea that diurnal regulation of a specific K^+ conductance in VIP neurons underlies the daily changes in neuronal activity in the SCN. Ongoing experiments, combining *in vivo* shRNA-mediated "knockdown" with whole-cell voltage clamp recordings in *in vitro* slices are focused on identifying the subthreshold K^+ channel(s) that control the daily rhythms in spontaneous firing of VIP neurons in the SCN.

Disclosures: T. Hermanstyne: None. **C.L. Simms:** None. **E.D. Herzog:** None. **J.M. Nerbonne:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH HD042634

Title: VIP neurons in the suprachiasmatic nucleus of neonatal mice with disrupted fibroblast growth factor signaling

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Abstract: Fibroblast growth factor (Fgf) 8 and its cognate receptor, Fgfr1, are essential for the development of multiple brain regions. Previous studies from our laboratory showed that reduced Fgf8 signaling led to the malformation of neuroendocrine nuclei that originated within the

diencephalon, including the oxytocin system in both the paraventricular (PVN) and supraoptic (SON) nuclei. To further understand the role of Fgf8 in the development of other hypothalamic nuclei, we examined if Fgf8 and Fgfr1 deficiencies also impact the integrity of the suprachiasmatic nuclei (SCN). The SCN are principal regulators of the organism's circadian rhythm and consist of neurons that produce vasoactive intestinal peptide (VIP) as main input neurons. The objective of this study is to examine the number of VIP neurons in the SCN of postnatal day (PN) 0 mice hypomorphic for Fgf8, Fgfr1, or both Fgf8 and Fgfr1. Brains were fixed in 4% paraformaldehyde, sectioned in a cryostat, and processed for VIP immunohistochemistry. The number of VIP-immunoreactive (ir) neurons was then quantified in the SCN. Double homozygous (DHom) mice that were homozygous for both Fgf8 and Fgfr1 deficiencies showed a conspicuous absence of SCN as well as SCN VIP-ir neurons. Fgfr1 heterozygous mice, however, showed increased numbers of VIP-ir neurons when compared to wild type (WT) mice, whereas Fgf8 heterozygous mice showed decreased numbers of VIP-ir neurons compared to WT. These data suggest that deficiencies in Fgf8 and Fgfr1 can impact the structural integrity of the SCN via multiple mechanisms.

Disclosures: A. Miller: None. **S. Kavanaugh:** None. **P. Tsai:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: Concordia University VPRGS Team Start-up/Accelerator Grant Program

Title: Initial characterization of circadian phenotype in ApoE knockout mice

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Abstract: In humans, disruptions of circadian rhythms have been linked to Alzheimer's disease (AD). Such circadian disruptions are often associated with sleep, endocrine and metabolic abnormalities that contribute further to poor health, stress and loss of quality of life in affected individuals. Apolipoprotein E (ApoE), a major regulator of lipid homeostasis, has been recognized as a key player in a wide range of central nervous system processes and behaviors in

mammals. Genetic manipulations of ApoE in mice have been shown to affect brain development and plasticity, learning and memory, feeding, and responsiveness to stress. Importantly, one of the copies of the human ApoE gene (ApoE epsilon 4) is the primary genetic risk factor for the development of AD. Recent evidence indicates that deletion of ApoE in mice can affect the rhythms of expression of genes (Per2, Cry1 and Bmal1) that form the molecular feedback loop of the circadian clock. This finding implicates ApoE in the control of the circadian clock and raises the intriguing possibility that changes in the activity of ApoE might be the missing link between AD pathology and dysregulation of circadian clock physiology. The aim of this project was to initially characterize the effect of deletion of ApoE on circadian activity rhythms in ApoE knockout mice (ApoE(-)). To assess circadian behavioral rhythms, 2-4 months ApoE(-) and wild-type control mice (WT) were individually housed in cages equipped with running wheels, with free access to food and water, and their wheel-running activity was recorded continuously using a computerized data acquisition and analysis system. Mice lacking the ApoE gene (ApoE(-)) exhibit an anomalous circadian behavioral rhythm. Specifically, although ApoE(-) mice exhibit stable circadian behavioral (wheel-running) rhythms when housed under a 12h:12h light/dark (LD) cycle and no difference was found in the amount of activity compared to wild-type control mice (WT), they require significantly more time to adjust to large shifts (phase advance: 7 ± 1 days, phase delay: 8 ± 1 days) in the LD schedule compared to WT (phase advance: 3 ± 1 days, phase delay: 3 ± 1 days). Furthermore, the length of the free-running locomotor activity cycle, which reports the intrinsic speed of the master circadian clock (the suprachiasmatic nucleus, SCN), was significantly longer in the ApoE(-) mice compared with WT controls. These preliminary results show that genetic deletion of the ApoE gene affects fundamental aspects of the master brain clock in mice, and lend support to our hypothesis that ApoE may function as a molecular link between AD pathology and disruption of circadian clock physiology.

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Poster

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Title: A comparison of neuronal activity and CRY1 expression in the suprachiasmatic nucleus and subparaventricular zone of diurnal tree shrews

Authors: *L. M. HABLITZ[1], J. R. PAUL[2], L. A. MCCOLLUM[2], J. T. SIEGWART[3], R. C. ROBERTS[2], T. T. NORTON[3], K. L. GAMBLE[2]
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Abstract: Circadian rhythms, 24-hour cycles in biological and behavioral processes, are driven mainly by the suprachiasmatic nucleus (SCN) of the hypothalamus in mammals. Functionality of the SCN is thought to be independent of temporal activity phases (nocturnality or diurnality) of the animal, with higher action potential firing during the day. Previous reports comparing laboratory rats to diurnal grass rats (Arvicanthis niloticus) have identified the lateral subparaventricular zone (SPZ) as a potential regulator of diurnality. These studies show day-active grass rats have synchronized expression levels for c-Fos protein that are high during the day and low at night in both SCN and SPZ, whereas night-active laboratory rats exhibit c-Fos expression that is out of phase between the SCN and SPZ. However, no studies to date have looked at the intrinsic firing rates of neurons in both brain regions during the day versus the night in a diurnal animal. Here, we utilized the exclusively diurnal tree shrew (Tupaia glis belangeri), and performed both loose patch electrophysiology and immunohistochemistry for CRY1 protein expression to evaluate the relationship of clock gene expression and neuronal activity between the SPZ and SCN. We show that the SPZ and SCN have significantly higher firing rates in the day (mean ± SEM, SCN: 5.61 ± 0.49Hz, SPZ: 5.56 ± 0.49Hz) compared to the night (SCN: 3.78 ± 0.31Hz, SPZ: 2.58 ± 0.28Hz). Preliminary immunohistochemistry supports this result, with higher levels of CRY1 at ZT18 in both the SCN and SPZ compared to ZT6. In conclusion, we show for the first time that diurnal tree shrews have higher firing rates in both the SCN and SPZ during the day, corresponding with lower levels of CRY1 at ZT6 compared to ZT18. This study provides further insight into how nocturnal and diurnal animals have similar SCN temporal function, yet can alter timing of their active phase, potentially using downstream targets such as the SPZ as a nocturnal/diurnal “switch”.

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Poster

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Title: Optogenetic manipulation of suprachiasmatic nuclei neurons modulates circadian behavior

Authors: *M. TACKENBERG[1], J. R. JONES[1], D. G. MCMAHON[1,2]
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Abstract: Circadian rhythms in mammals are orchestrated by the suprachiasmatic nuclei (SCN) of the hypothalamus. The circadian pacemaker is remarkable for both its adaptability and its stability, responding to changes in light/dark (LD) cycle by shifting its internal phase. These light-induced phase changes of the overall pacemaker are generated through corresponding changes in gene oscillations and firing rate rhythms within each SCN neuron. We are able to induce changes in SCN neuron firing rate through optogenetic stimulation of channelrhodopsin (ChR2) specifically targeted to SCN neurons in mice. Hour-long blue light stimulation reliably induced phase shifts in circadian locomotor behavior in experimental animals but failed to cause changes in control animals. The magnitude and direction of changes in phase of behavioral rhythms that we observe post-stimulation correspond to the shifts predicted for external light pulses delivered at the same phase. Inhibitory drive to these same neurons using yellow-light activation of halorhodopsin (NpHR) was also able to produce phase shifts *in vivo*, though these shifts did not correspond to predicted light pulse-induced changes and may align with dark-pulse induced shifts. Additionally, mice reared in constant bright light to induce behavioral arrhythmicity were stimulated once with blue light to induce firing within the SCN, leading to the generation of circadian rhythms in locomotion. These results indicate that artificial generation of action potentials within the SCN can effectively reorganize the circadian pacemaker. Used *in vitro*, this technique allows for the replication of light-induced changes to firing rate, as well as for toxin-free inhibition of firing. Combined with bioluminescent clock gene expression reporters such as PER2::LUC, network dynamics of clock components in intact SCN slices can be observed before, during, and after optogenetic manipulation. The ability to manipulate SCN firing rate through the stimulation of ChR2 and inhibition via NpHR is

therefore an important tool for the investigation of the role of firing rate in the SCN on core circadian behavior.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Program#/Poster#: 454.10/RR7

Topic: E.08. Biological Rhythms and Sleep

Title: Influence of light condition to physiological response and neurotransmitters

Authors: ***T. MATSUMURA**[1], H. NAKAGAWA[2], K. SUZUKI[2], C. NINOMIYA[2], S. YANAGITA[3], H. HASEGAWA[4], T. ISHIWATA[2]
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Abstract: Our recent society is said to be a stressed society, and it is essential to manage stress appropriately. The living environment for many people has become wealthy and it is easy to live comfortably, but this has had negative effects on life style, leading to an increase in patients with mental illnesses, including depression. Brain neurotransmitters, such as serotonin (5-HT), dopamine (DA), and noradrenaline (NA), control activity and influence mental states. There have been reports that irregular light/dark cycles cause a decrease in learning ability, and an increase in depression-like behavior. However, the relationships between circadian rhythm, physiological indices, stress indices, and levels of neurotransmitters remain unknown. We studied how an irregular light/dark cycle influences physiological and stress indices, and measured the levels of 5-HT, DA, and NA in the brains of rats. Male Wistar rats were maintained in two different environments (12-h/12-h or 6-h/6-h light/dark cycles) with food and water ad libitum. There were three groups: a control group (n = 10), maintained with a 12-h/12-h light/dark cycle for 1 month; and irregular light cycle groups, maintained with a 6-h/6-h light/dark cycle for 2 weeks (n = 5), or one month (n = 10). After the specified periods, the rats were sacrificed. The frontal cortex (FC), caudate putamen (CPU), preoptic area (PO), paraventricular hypothalamus (PVN), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), and posterior hypothalamus (PH), hippocampus (CA1), amygdala (Amy), ventral tegmental area (VTA), locus coeruleus (LC), substantia nigra (SNr), median raphe (MR),

dorsal raphe (DR), and suprachiasmatic nucleus (SCN) were immediately dissected out and sliced into 300-μm-thick sections. Samples were cut from each area using a disposable 1-mm-diameter biopsy punch. After washing with Ringer's solution, tissues were ground gently using a disposable pestle in a microtube containing 200 mL ice-cold 0.1 M perchloric acid. The levels of 5-HT, DA, and NA in each area were analyzed using high-performance liquid chromatography. Irregular light/dark cycle rats showed decreased levels of 5-HT in the SCN compared to the control group, and their activity was decreased in an open field test. DA and NA were not influenced by a change in circadian rhythm, and only 5-HT was influenced by this change. The decrease in 5-HT caused depression-like behavior.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Title: Copper in the suprachiasmatic nucleus clock: Exploring interactions between Cu transporters, homeostasis, and circadian neuronal activity *in vitro*

Authors: *Y. YAMADA, R. A. PROSSER
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Abstract: Glutamate (Glu) activation of the NMDA receptor coupled with TrkB signaling resets the phase of circadian activity in the suprachiasmatic nucleus (SCN), the master clock in the hypothalamus. Different pathways are activated downstream of Glu and TrkB signaling depending on time of day and induce shifts in SCN neuronal activity rhythms (phase shifts). These Glu-dependent phase shifts require increases in MAPK/ERK activity, but regulatory factors prevent increased MAPK/ERK signaling by Glu during the day. Copper (Cu) is an essential trace element and has diverse roles in brain function. Cu modulates the activity of the NMDA receptor and is a cofactor for several important enzymes in the brain, including MEK1 (MAPK kinase). Recent studies have demonstrated that: Cu increases MAPK/ERK activation; and decreasing Cu availability by chelation or genetic manipulation of the Cu importer, Copper transporter-1 (CTR1), decreases MAPK/ERK activity. Several brain regions with high Cu concentrations have been identified including the hypothalamus, where synaptosomal Cu release

has been reported. In addition, Cu release in neurons requires the Cu transporter ATP7A. Using acute, hypothalamic slices of adult, male C57Bl/6 mice, we are investigating the role of Cu in SCN neuronal activity rhythms and whether mechanisms underlying phase shifts and circadian physiology interact with Cu homeostasis. Our preliminary measurements of Cu levels in hypothalamic tissue using ICP-MS are consistent with the literature, supporting a role for Cu in this brain area. Furthermore, our immunoblot data siggests circadian ATP7A expression, which could indicate circadian changes in Cu homeostasis and possibly even in the synaptic release of Cu. Previously, we demonstrated by *in vitro* extracellular recordings that bath-application of Cu at night induces phase shifts in SCN activity rhythms. Cu-induced phase shifts are not dependent on NMDA receptors but are inhibited by co-treatment with the MEK inhibitor U0126, consistent with Cu-induced MAPK/ERK activation. As shown here, CTR1 is expressed in the SCN, and so Cu import could play a role in circadian MAPK/ERK regulation in the SCN. On the other hand, we have shown that the Cu-specific chelator, tetrathiomolybdate (TTM), induces phase shifts *in vitro* when applied during the night or at midday. TTM-induced nighttime phase shifts are not blocked by the MEK inhibitor or the PI3K/mTOR inhibitor LY294002. We plan to investigate activation of other pathways in phase shifts and the expression patterns of other Cu proteins.

Disclosures: Y. Yamada: None. **R.A. Prosser:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

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Title: ATP signaling synchronizes ensemble rhythms among suprachiasmatic nucleus astrocytes

Authors: *A. C. CAMACHO[1], A. D. WOMAC[1], N. NEUENDORFF[2], Y. F. FARNELL[2], D. J. EARNEST[2], M. J. ZORAN[1]
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Abstract: The suprachiasmatic nuclei (SCN) of the hypothalamus contain thousands of neurons and glial cells that function in coordinating system-level physiological rhythms that are entrained to environmental light cues. In the SCN of the rat, extracellular ATP accumulates in a circadian manner during light/dark cycles and in constant darkness, with peak levels at night and

subjective night. ATP is released rhythmically from astrocytes in immortalized rat SCN2.2 cell lines, where disruption of purinergic signaling pathways disrupts the period and amplitude of rhythms in both ATP release and clock gene expression. Little known about the mechanisms that mediate synchronization of astrocytes, in the SCN or in other brain regions. We have studied the role of ATP signaling in the process of astrocyte synchronization. First, mouse SCN astrocytes possess an intrinsic rhythmicity in purinergic sensitivity, as assayed by ATP-induced calcium transients, and this rhythm in ATP sensitivity is absent in Per1ldcPer2ldc double mutant astrocytes with defective molecular clock mechanisms. Second, pharmacological blockade of purinergic signaling, with ayprase-mediated ATP hydrolysis, causes a significant reduction in period of clock gene expression rhythms, as determined by bioluminescence reporting in SCN astrocytes derived from mPerluc mice. Thirdly, exogenous application of ATP at specific time points causes phase shifts or arrhythmicity in ATP release rhythms of SCN2.2 astrocytes. Therefore, astrocytes of the mammalian SCN rhythmically release ATP and that rhythmic ATP signaling mediates synchronization of multiple neurophysiological events in these glial cells.

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Poster

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Support: Univ. Tennessee Donald Akers Research Fellowship

Title: LRP1 modulates phase shifting in the mammalian circadian clock partly independent of interactions with tPA

Authors: *J. COOPER, R. A. PROSSER
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Abstract: The mammalian circadian clock in the suprachiasmatic nucleus (SCN) exhibits daily rhythms in neuronal activity and synaptic plasticity, and its phase regulation involves both extracellular and intracellular mechanisms. Neurotrophins and extracellular proteases regulate synaptic plasticity throughout the brain, while in the SCN they regulate the ability of the circadian clock to phase shift. Photic regulated clock phase shifting depends on glutamate release

from retinal ganglion cells to activate NMDA receptors; *in vitro* glutamate application mimics light-induced phase shifting. These phase shifts require concurrent activation of TrkB receptors by brain-derived neurotrophic factor (BDNF). We previously showed that tissue-type plasminogen activator (tPA) proteolytic activity regulates glutamate-induced phase shifts *in vitro*: tPA cleaves plasminogen into plasmin; plasmin cleaves pro-BDNF into its active form m(ature) BDNF; and mBDNF binds TrkB receptors allowing clock phase shifts (Mou et al 2009). However, much remains unknown about molecular mechanisms that gate phase shifting. Low density lipoprotein receptor-related protein 1 (LRP1) regulates diverse functions in the brain and provides unique ways for cells to control and respond to their extracellular environment. LRP1can influence neuronal signaling through interactions with specific ligands, including tPA. In this study we address the hypothesis that LRP1 is involved in circadian clock phase regulation, and that this may be via interactions with tPA. SCN brain slices from adult male C57BL/6 mice were treated with glutamate (1mM) +/- LRP1 inhibitors (500nM RAP or 75µg/mL anti-LRP1 antibody) at ZT 16 or ZT 23 (where ZT 0=lights-on in the animal colony) for 10 minutes. The following day we recorded SCN single-unit neuronal activity (SUA) to determine the time of peak activity. Concurrent application of RAP or anti-LRP1 with glutamate prevents the normal shifts, while RAP alone has no effect on clock phase. We investigated the interrelationship of tPA and LRP1 using tPA knockout (KO) mice, B6.129S2-Plattm1Mlg/J. First we characterized the SUA rhythm in control tPA KO brain slices and after glutamate treatment. SCN slices from adult male tPA KO mice exhibit entrained neuronal activity rhythms, and 10µM glutamate at ZT16 and ZT23 induces phase delays and phase advances, respectively. Thus, tPA KO mice do not exhibit severe deficiencies in clock phase regulation, possibly reflecting redundant mBDNF-generating pathways. Finally, RAP inhibits glutamate phase resetting in tPA KO slices, indicating LRP1 may be able to influence the clock independently of tPA.

Disclosures: J. Cooper: None. **R.A. Prosser:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Title: Inhibiting matrix metalloproteinases 2 and 9 phase shifts neuronal activity rhythms in the suprachiasmatic nucleus

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Abstract: Photic entrainment of mammalian circadian rhythms occurs within the suprachiasmatic nucleus (SCN), where the master clock is localized. During the early subjective night, exposure to light or *in vitro* application of glutamate delays clock phase, which is seen *in vitro* as a shift in peak neuronal activity. In addition to complex intracellular processes, a variety of extracellular proteins regulate photic/glutamate phase-shifting processes in the SCN. Notably, proteolytic conversion pro-Brain Derived Neurotrophic Factor (pBDNF) to its mature form (mBDNF) allows activation of TrkB receptors, a necessary step for these phase shifts (Mou et al 2009). That study also implicated tissue-type plasminogen activator (tPA) in this proteolytic step. Matrix metalloproteinases, MMP2 and MMP9, generally serve as extracellular matrix remodelers (ECM), but have also been shown to convert pBDNF to mBDNF in neuronal cell cultures. Based on these data, we hypothesized inhibition of MMP2/9 would block glutamate-induced phase delays. Single-unit, extracellular recordings were taken from acute SCN brain slices prepared from adult, male C57BL/6 mice. Slices were maintained for two days *in vitro* and were exposed to bath-applied drugs at Zeitgeber time (ZT) 16 (ZT 0=lights on and ZT 12=lights-off in the animal colony) on day one. Spontaneous activity of SCN neurons was recorded the day following drug treatment. Contrary to our initial hypothesis, *in vitro* application of the MMP2/MMP9 inhibitor, BiPS ((2R)-[(4-Biphenylylsulfonyl)amino]-N-hydroxy-3-phenylpropionamide; 100nM-10uM), alone for 50 min at ZT 16 induced 2-3 h delays, similar to the effects of glutamate. Additionally, phase shifts induced by BiPS were blocked by co-application of an NMDAR antagonist [(2 R)-amino-5-phosphonopentanoate; AP5]. Preliminary data suggests that a BiPS induced delay persists when TrkB activiation is blocked using both K-252A, a TrkB antagonist, and -2 antiplasmin, a plasmin inhibitor. Preliminary results from western blots have detected MMP2 and MMP9 in SCN slices. Interestingly, this data suggests MMP9 may be expressed higher at night than during the day. However, as MMP2/9 are zymogens, data gathered from gelatin zymography technique will show what ratio of expressed protein is active. Currently, we are investigating how MMP2/MMP9 modulates NMDAR signaling and whether BiPS induces phase advances when applied at ZT 23. Combining the aforementioned studies with temporal assessments of MMP2/MMP9 activity and expression should clarify the role(s) of these proteases in the SCN.

Disclosures: K.E. Abrahamsson: None. **R.A. Prosser:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: E.08. Biological Rhythms and Sleep

Support: UMDNJ Foundation

Osteopathic Heritage Research Foundation

Title: A possible role for the Neuronal calcium protein, neurocalcin delta, in photoentrainment of circadian rhythms

Authors: *J. ZHANG[1], R. SWANSON[2], A. KRISHNAN[1], V. VENKATARAMAN[1]
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Abstract: The free-running clock in mammals has a periodicity of about 24 hours. It is entrained into a 24-hour clock by many stimuli. The predominant one among these is light. Results from this laboratory indicate that the neuronal calcium sensor protein neurocalcin delta (NCALD) contributes to the photoentrainment of the circadian clock. Based on results obtained from immortalized SCN progenitor cells, both the mRNA and protein levels for NCALD exhibit robust oscillations. These oscillations are also observed in rats and mice at the mRNA level (determined by qPCR) and protein level (determined by immunostaining and/or Western blotting). A characteristic feature of the NCS proteins is the calcium-myristoyl switch, which promotes calcium-dependent movement of the proteins to the membranes. It is our hypothesis that the switch is central to the role of NCALD in photoentrainment. Using wild-type and mutant NCALD constructs fluorescently tagged with YFP, we demonstrate that agents that alter cellular calcium cause translocation of NCALD within the cell and that the calcium-myristoyl switch is critical for the translocation.

Disclosures: J. Zhang: None. **A. Krishnan:** None. **V. Venkataraman:** None. **R. Swanson:** None.

Poster

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Program#/Poster#: 454.16/RR13

Topic: E.08. Biological Rhythms and Sleep

Support: KAKENHI 22300108

Title: Cytosolic calcium mobilizations and resetting of molecular clock oscillations via M3 muscarinic receptors in human retinal pigment cells

Authors: *M. IKEDA[1], H. AKECHI[1], M. TAKEDA[1], K. TAKEUCHI[2], T. EBISAWA[3]
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Abstract: Molecular basis of circadian clock has been analyzed in a wide variety of organisms, yet understanding of the human clock system remains challenging. Here, we generated a cell line (hRPE-YC) from human retinal pigmental epithelium, which stably co-express reporters for molecular clock oscillations (Bmal1-luc) and intracellular Ca2+ concentrations (YC3.6). The hRPE-YC cells represented Bmal1 transcriptional rhythms. Also, the hRPE-YC cells represented spontaneous Ca2+ spiking rhythms, having no apparent circadian rhythms in the baseline Ca2+ levels. Receptors triggering intracellular Ca2+ elevations were randomly screened and we found expression of muscarinic receptors in hRPE-YC cells. Real time RT-PCR and series of pharmacological studies further demonstrated functional M3 receptor expressions regardless of circadian phases. A muscarinic agonist, carbachol, phase-shifted Bmal1 oscillations, and formed a type 1 phase-response curve. On the other hand, light pulse exposure failed to induce corresponding phase-shifts in hRPE-YC cells. This may be due to relatively small expression of cryptochrome or by lack of melanopsin in these cells. Since it has recently shown that photoreceptor outer segments (OS) could communicate with RPE microvilli using acetylcholine as a transmitter, such cholinergic control from photoreceptor cells may determine the phase of RPE cells. Also, since RPE phagocytosis determines disk shedding in OS, it is possible that molecular clock oscillations in RPE cells may involve the timing of disk shedding. In conclusion, the present results provide a cellular model to understand molecular clock oscillations, regulations, and de novo functions of cholinergic systems in human eyes.

Disclosures: **M. Ikeda:** None. **H. Akechi:** None. **T. Ebisawa:** None. **K. Takeuchi:** None. **M. Takeda:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Program#/Poster#: 454.17/RR14

Topic: E.08. Biological Rhythms and Sleep

Title: Effect of sodium light on circadian rhythms in mouse and rat

Authors: *X. CHEN, G. J. DEMARCO
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Abstract: Studies in nocturnal rodents often need to be performed during their active phase in darkness. Low-pressure sodium lights emit light visible to humans but not rodents and has been used to conduct studies and animal welfare checks in the dark phase. This study tested the hypothesis that sodium lighting would influence in rodents. Body temperature and locomotor activity were the circadian endpoints quantitated and captured from telemetry implanted C57BL lean and diet induced obese (DIO) mice, and Sprague-Dawley rats. A 2-hour sodium light pulse within the dark phase, which mimicked a procedure, disrupted entrained circadian rhythms in mice. When rats were exposed to a sodium light pulse under free running conditions (constant darkness), it caused phase shifts that were similar to those seen after a white light pulse. Mice and rats placed under a 12:12-hour sodium light:dark cycle, entrained to sodium light similar to entrainment under standard lighting conditions. Mice placed under a 12:12-hour sodium light:white light cycle, entrained to the white light and had altered activity and body temperature profiles in the sodium light phase. The effect of sodium light on DIO mice was similar to that seen in C57BL lean mice. These data suggest that sodium lighting is not the equivalent of complete darkness and it can influence the circadian timing system in rodents.

Disclosures: X. Chen: None. **G.J. DeMarco:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: DOE FG02-05CH11318

NSF IOS-1022050

NIH NS078220

Title: The effect of long-term non-contiguous blockade of $GABA_A$ receptors in the SCN on light-induced phase shifts

Authors: P. WALKER, II[1], J. S. BROWN[1], E. RUSSOM[1], A. PEGGINS[1], H. E. ALBERS[2,3], *D. L. HUMMER[1,3]
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Abstract: Mammalian circadian rhythms are generated and coordinated by a biological clock located in the suprachiasmatic nucleus of the anterior hypothalamus (SCN). Light acts at dawn and dusk to adjust the phase of these endogenously generated rhythms, permitting organisms to synchronize their physiology and behavior with a 24-hour cyclical environment. Light exposure results in sustained changes in both neural activity and gene expression within the SCN (Yan & Silver, 2002; Kuhlman et al., 2003; Hamada *et al*., 2004; LeSauter et al., 2011); however, the role of these sustained events in circadian entrainment has not received attention. Our hypothesis is that sustained activation of $GABA_A$ receptors in the SCN mediates the ability of light to shift the biological clock. Our lab has shown that 6 hours of $GABA_A$ antagonist administration into the SCN inhibits the ability of light to phase delay the clock during early subjective night. The current experiment was conducted to determine whether or not 6 *contiguous* hours of $GABA_A$ antagonist administration is required to inhibit light-induced phase delays. Male Syrian hamsters were implanted with a guide cannula aimed at the SCN, introduced to running wheels, and allowed to establish a stable, free-running activity rhythm in constant darkness. Hamsters then received a 15-minute light pulse at CT13.5, followed by 8 consecutive hourly microinjections into the SCN between CT14.5 and CT21.5. Animals received microinjections containing either a $GABA_A$ antagonist, bicuculline (B), or saline (S) in one of two regimens: (1) 8 injections of saline (S-S-S-S-S-S-S-S), or (2) 3 injections of bicuculline, followed by 2 injections of saline, followed by 3 additional injections of bicuculline (B-B-B-S-S-B-B-B). The size of the phase shift was determined by comparing the onsets of activity on the day of treatment, predicted from the daily onsets of activity before and after treatment. Preliminary data indicate that light-induced phase delays do not differ between hamsters treated with bicuculline in a non-contiguous manner and saline treated controls. These data are consistent with the prediction that 6 contiguous hours of $GABA_A$ receptor blockade in the SCN is required to inhibit light-induced phase delays.

Disclosures: P. Walker: None. **J.S. Brown:** None. **E. Russom:** None. **A. Peggins:** None. **H.E. Albers:** None. **D.L. Hummer:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: IOS-1021957

Title: Response of circadian locomotor activity rhythms to injections of the CB1 agonist Win 55, 212-2

Authors: *A. C. KLEIN[1], E. M. MINTZ[1,2]
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Abstract: Circadian rhythms of physiology and behavior are driven by a clock located in the suprachiasmatic nucleus of the hypothalamus. Cannabinoid receptors are expressed in the SCN, however, our knowledge of their role in clock function is limited. Previous studies have suggested that exogenous cannabinoids can act on the SCN to inhibit the phase shifting effects of light in mice, but focused on a narrow phase of the circadian cycle. To begin to address the question of the role of cannabinoid signaling in circadian rhythm regulation, we examined the response of the circadian locomotor activity rhythm to systemic injections of Win 55,212-2, a cannabinoid receptor 1 agonist. Adult male C57BL/6J mice were housed in constant dark and given i.p. injections (3 mg/kg) of Win 55,212-2 or vehicle controls at times throughout the circadian cycle. The results were used to compile a phase response curve to Win 55,212-2, and binned into 2-hr increments for statistical comparison by ANOVA. There was a significant interaction between time of injection and the injection, indicating that an effect of the drug varied as a function of circadian time. The major difference occurred at circadian time 12 (the onset of subjective night), where Win 55,212-2 induced a modest phase advance (0.43 ± 0.07 hrs) that was significantly different from the small phase delay (-0.46 ± 0.22) that occurred after saline. At all other phases tested, Win 55,212-2 produced, on average, a small phase delay. Future experiments will attempt to determine the mechanism by which systemic administration of cannabinoid agonists affect circadian clock function.

Disclosures: **A.C. Klein:** None. **E.M. Mintz:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Title: Response of the orexin, mch, npy and alpha msh neuronal population to different food schedules

Authors: *O. RAMÍREZ PLASCENCIA[1,2], G. MARTEL-GALLEGOS[1], C. ESCOBAR[3], N. SADERI[1], R. SALGADO-DELGADO[1,2]
[1]Fac. of Sci., [2]Med. Sch., Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico; [3]Med. Sch., Univ. Nacional Autónoma de México, Mexico city, Mexico

Abstract: Temporal regulation of behavior and metabolism in mammals is important for health. The synchrony between the biological clock, the Suprachiasmatic Nucleus (SCN), and external temporal cues keeps a dynamic homeostasis that controls body physiology. However, the modern life style promotes diverse situations that induced activity and food intake during the rest phase, causing an Internal Desynchronization (ID). The ID is related to some metabolic alterations, such as obesity and metabolic syndrome. It has been suggested that the neural circuitry in Central Nervous System responsible for metabolic and time regulations could be playing an important role in the alterations induced by ID. IN the hypothalamus, there are two nuclei that are pivotal for the integration of metabolic and time information: the Lateral Hypothalamus (LH) and PeriFornical zone (PeF), both expressing two neuropeptides: Orexin (ORX), which is essential for wakefulness and have a catabolic effect, and Melanin-Concentrating Hormone (MCH), which is involved in sleep onset and have an anabolic effect. These nuclei receive time information directly from the SCN and metabolic information mainly by the hypothalamic Arcuate nucleus (ARC). In the latter there are two neural populations able to detect metabolic cues: Neuropeptide Y (NPY) neurons, which increase food intake and decrease the metabolic rate, and alpha Melanocyte−Stimulating Hormone (α−MSH) neurons, which have an opposite effect. To know which specific neural population in this circuitry is affected by ID we use male Wistar rats which were assigned to 3 groups: ad Libitum (AL), who had free access to food; fed during rest phase (FRP); and fed during active phase (FAP). After 21 days under this feeding schedules, animals were sacrificed on 4 different temporal points: ZT0, ZT6, ZT12 and ZT18. ORX, MCH, NPY and αMSH neurons, co−expressing c−Fos, were identified by selective protein immunohistochemistry. Results show that in the FRP rats ORX neurons are active during the day, which is the opposite of what occurs in AL and FAP groups. MCH neurons decrease their

activity only in the anterior LH of FRP animals in comparison to AL and FAP rats. In addition, we found that in in the ARC α−MSH are active during the phase in which animals eat. Finally, the activity of NPY neurons is not affected by feeding schedule. In conclusion, results show that a protocol of ID caused by food restriction to the rest phase alters the pattern of expression of neuropeptides involved in the control of energy homeostasis, such as ORX and α-MSH. We suggest that these changes could be involved in the metabolic impairment described for the ID.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF Grant IOS 11-18792

Title: Sex differences in the response to exposure to light at night and high fat diet during early life

Authors: *Y. M. CISSE, R. J. NELSON
Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: The adoption of electric lights has brought about obvious advantages to schedule flexibility and productivity, but at a cost that is just now becoming appreciated. Light is one of the most potent signals to the circadian system; thus, exposure to light at night (LAN), disrupts synchronization to the natural light-dark cycles, and dampens central and peripheral clock gene expression. The circadian system regulates metabolism, driving physiologically-relevant oscillations in gene transcription and downstream metabolic hormones and enzymes. Exposure to LAN has been associated with increased body mass and altered feeding rhythms in adult mice. High fat diet (HFD) also disrupts behavioral and molecular rhythms in adult mice. When combined with LAN, HFD exacerbates body mass gain and impairs glucose processing; however, the effect of these two factors during development remains unspecified. Early life is a critical time for the development of endogenous circadian rhythms, as well as metabolic priming. Because disruption of early postnatal clock gene expression precedes adult obesity phenotypes, we predicted that disruption of circadian rhythmicity through LAN alone or in conjunction with HFD primes for metabolic dysfunction in adulthood. Mice were bred in our lab and litters were normalized to 10 pups (control litters: CL) or reduced to 3 pups (small litter: SL). CL mice were

weaned onto chow diet, whereas SL mice were given HFD to maintain a lifelong HFD condition. Mice from both groups were also assigned to different light conditions; half were maintained in a standard light-dark (LD) cycle or exposed to nightly dim (5 lux) light (LAN). After four weeks in respective light conditions, food intake and locomotor activity were assessed. At nine weeks of age mice underwent either glucose tolerance testing or hippocampal, fat, and liver tissues were collected for qPCR. Female mice exposed to lifelong HFD did not exhibit hyperphagia or impaired glucose tolerance despite weight gain relative to chow fed mice. Females exposed to LAN and HFD reduced body mass at Weeks 5 and 6 despite no increase in daytime food intake. Male mice exhibited HFD induced hyperphagia and impaired glucose tolerance. Males exposed to LAN and HFD increased daytime food intake despite no total increase in calories consumed between HFD groups. Unlike adults, LAN males decreased HFD-induced weight gain. These results suggest that LAN exposure starting in early life disrupts metabolism in a separate, HFD dependent, and sex specific manner than that observed in adults. Additional studies are underway to elucidate the divergent weight phenotypes observed at different ages of exposure to LAN.

Disclosures: Y.M. Cisse: None. **R.J. Nelson:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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CONACYTI010/152/2014 C-133/2014

Title: Circadian nursing synchronizes septum, bed nucleus of the stria terminalis and preoptic area in rabbit does

Authors: J. AGUIRRE-CHIÑAS[1], *E. MEZA[4], S. WALISZEWSKI[2], R. C. ZEPEDA[2], M. CABA[2,3]
[1]Doctorado en Ciencias Biomédicas, [2]Ctr. de Investigaciones Biomédicas, Univ. Veracruzana, Xalapa, Mexico; [3]Posgrado en Neuroetología, Univ. Veracruzana, Xalapa, Veracruz, Mexico; [4]Univ. Veracr, Xalapa, Mexico

Abstract: In the rabbit, maternal behavior after parturition is restricted to a brief nursing period of less than 5 min each day with circadian periodicity. Previously we demonstrated that this short event is a strong signal that synchronizes oxytocin and dopaminergic producing cells in the brain. The activation of these neuroendocrine cells is very important in considering that milk needs to be produced and ejected with circadian periodicity. However the synchronization of other brain regions important for the behavioral expression of maternal behavior of this species had not been explored. Here we hypothesized that circadian nursing synchronizes the septal area, bed nucleus of the stria terminalis (BNST) and preoptic area (POA) in the lactating doe. To this aim we explored by immunohistochemistry PER1 protein, the product of the Per1 clock gene in the dorsal, medial and ventral Septum, in the anterior division of the BNST, in the POA and in the suprachiasmatic nucleus (SCN), the master circadian clock. Does were in 12 h light/dark condition (07:00, lights on, = ZT0), nursing was scheduled either at ZT19 or ZT03 and subjects were perfused every 4 h through a complete 24-h cycle at postpartum day 7. To determine persistence of possible oscillations an additional group of does was not permitted to nurse for 24 or 48 h. Nonpregnant, nonlactating does were used as controls. In control does only the SCN show a clear rhythm with maximal PER1 expression at ZT11 and also in nursing does. On the contrary, in the dorsal and ventral, but not medial, Septum, in the BNST and in the POA nursing induces a rhythm of PER1 that reaches a peak 8 h after scheduled nursing. This effect was nursing dependent as their deprivation reduces the number of PER1 at the time of peak expression in most regions explored except in the POA. We conclude that the three telencephalic areas analyzed participate in the expression of maternal behavior in the rabbit and suggest that the POA plays a main role as the number of PER1 protein is mostly unaffected by lack of nursing.

Disclosures: J. Aguirre-Chiñas: None. **E. Meza:** None. **S. Waliszewski:** None. **M. Caba:** None. **R.C. Zepeda:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF IOS-1021957

Title: Novel responses of daily wheel-running activity rhythms to restricted feeding cycles in mice

Authors: A. RASTOGI[1], J. A. MURPHY[1], *E. M. MINTZ[1,2]
[1]Biol. Sci., [2]Biomed. Sci., Kent State Univ., Kent, OH

Abstract: In response to food being made available for only a few hours each day, mice display increased locomotor activity in the hours preceding food presentation. This food anticipatory activity (FAA) continues as long as the food restriction is maintained, but disappears quickly once ad libitum feeding conditions are restored. To study the mechanisms underlying FAA, two experiments were performed, where we placed mice in conditions either in single phase restricted food (RF) and free food (FF) cycles, or in alternating RF (constant) and FF (variable) cycles in multiple phases. The present study was performed with two genotypes, memory deficient tissue-type plasminogen activator knock out ($tPA^{-/-}$) male mice and wild type (WT, C57BL/6J) males. $tPA^{-/-}$ mice are severely deficient in long-term potentiation, long-term depression, and hippocampal-based learning and memory tasks. We have previously shown that these mice show increased FAA. Initially, mice were individually maintained in 12L:12D photoperiod with FF. After entraining to LD conditions, in the first experiment we placed mice (n=6) either in RF (ZT6-10; ZT0: lights onset) or continue in FF cycles, and perfused them after 1 week at ZT5. In second experiment, two groups (control and experimental; n=6) had a week of RF (ZT6-10). In control groups, all RF phases were followed with 3 days (constant) of FF, while in experimental groups, RF phases were followed with 3, 6 and 9 days FF cycles (variable), sequentially. Animals were perfused at ZT5 after 1st RF cycle at the end of the experiment. Behavioral results suggest that mice lose weight after first RF bout, but not in subsequent bouts, showing adaptation to RF even with intervening FF periods. During subsequent RF bouts, $tPA^{-/-}$ mice recover FAA more quickly than WT, suggesting that this is not the result of hippocampal-dependent learning. Our Fos-immunohistochemistry data suggests that in experiment 1, in response to RF, Fos-immunoreactivity in the dorsomedial hypothalamus and the arcuate nucleus increases several fold relative to FF condition. In experiment 2, high Fos immunoreactivity was visible in the DMH, with increased activity in the control group as compared to experimental group, but in the arcuate nucleus Fos activity was very low or undetectable in both groups. Taken together, these data suggest that underlying mechanisms of FAA regulation may be exposed in $tPA^{-/-}$ mice.

Disclosures: A. Rastogi: None. **J.A. Murphy:** None. **E.M. Mintz:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF IOS-1021957

Title: Food anticipatory activity is regulated by tissue plasminogen activator and is influenced by biological sex

Authors: ***J. A. MURPHY**[1], L. E. MORELAND[1], E. M. MINTZ[1,2]
[1]Biol. Sci., [2]Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: Circadian rhythms of physiology and behavior are driven by a circadian clock located in the suprachiasmatic nucleus of the hypothalamus. This clock is synchronized to environmental day/night cycles by photic input, which is dependent on the presence of mature brain-derived neurotrophic factor (BDNF) in the SCN. Mature BDNF is produced by the enzyme plasmin, which is converted from plasminogen by the enzyme tissue plasminogen activator (tPA). In this study, we evaluated circadian function in mice lacking functional tPA. tPA(-/-) mice have normal circadian periods and phase shifts to light pulses, but show an increased proportion of daily wheel-running activity during the day and a slightly reduced overall level of wheel-running activity. When placed on daily cycles of restricted food availability, tPA(-/-) mice show much higher levels of food-anticipatory activity than wild type control mice. Despite the increased wheel-running activity in tPA(-/-) mice, neither they nor wild type mice entrain to 24-hour cycles of restricted food availability in constant darkness. If housed in a skeleton photoperiod (15 minutes of light bracketing a 12:12 light-dark cycle) the difference in FAA between genotypes is reduced but not lost. Furthermore, in the course of conducting these experiments, we observed that female mice show significantly smaller amounts of FAA than male mice. In tPA(-/-) mice, female mice show increased FAA compared to wildtype controls but FAA remains substantially lower than in male mice. If increased FAA in tPA(-/-) mice was associated with increased hunger, one would predict that these animals would have higher food intake on restricted feeding cycles, however, this was not the case. Overall, these data support a role for both tPA and biological sex in regulating food anticipatory activity.

Disclosures: J.A. Murphy: None. **L.E. Moreland:** None. **E.M. Mintz:** None.

Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: NSERC DG 311874

Title: Phase shifting the circadian clock with sleep deprivation: An EEG analysis of responders and non responders

Authors: P. BASU[1,2], J. MACDONELL[1], F. CORTESE[2], *M. C. ANTLE[1,2]
[1]Psychology, Univ. of Calgary, Calgary, AB, Canada; [2]Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Arousal-induced shifting of the circadian phase in response to sleep deprivation by gentle handling in Syrian hamsters is well known. However, only about two-thirds of animals tested exhibit phase shifts to the sleep deprivation procedure. The current study attempts to explore the arousal state of the brain, as measured by EEG, in both responders (i.e., those that shift to sleep deprivation) and non-responders, and thus correlate brain activity with post - sleep deprivation behavioral differences. Adult male Syrian hamsters (n=15) were maintained in a 14h:10h light-dark schedule and voluntary wheel-running activity was measured (Clocklab). Sleep deprivations were conducted using an Aschoff Type 2 design, where the animals transitioned from the light:dark cycle to constant dark at the start of the sleep deprivation procedure. Stably entrained animals were sleep deprived in dim red light (<1 lux) by gentle handling for 3 hours, while EEG was recorded. Subsequently, the animals were maintained under continuous darkness with access to voluntary wheels, and phase shifts were determined from the activity onsets. Animals exhibiting no post-sleep deprivation phase shift were designated non-responders and those that shifted; responders. These were compared on the basis of FFT analysis of power across Delta, Theta, Alpha and Beta frequencies. Non-responders exhibited a substantial peak in the low-alpha (7-9 Hz) range during sleep deprivation. Alpha activity is associated normally with drowsiness. These data indicate that it is not the loss of sleep per se that elicits the phase shifts, but rather that a sufficient level of arousal is necessary.

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Poster

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Topic: E.01. Neuroendocrine Processes

Support: DGAPA-PAPIIT IN215513-3.

Title: The effects of unilateral adrenalectomy to cyclic rats on corticosterone serum levels do not depend on the hour and day of the cycle when surgery was performed

Authors: *R. DOMINGUEZ, G. D. CORTÉS, J. C. MUÑOZ, C. C. SILVA, D. P. BENÍTEZ, M.-E. CRUZ, A. FLORES
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Abstract: The adrenals are the main source of progesterone during the estrous cycle, since bilateral adrenalectomy resulted in a significant decrease in progesterone concentration. The effects of unilateral adrenalectomy performed on estrus (E), diestrus-1 (D1), diestrus-2 (D2) showed differences depending on the *in situ* adrenal. The aim of present study was to analyze if a similar response occurs on corticosterone serum levels. Cyclic rats of the CIIZV strain, maintained under controlled light/dark cycle (lights on 05.00-19.00), on each day of the estrous cycle at 07:00, 09:00, 13:00, or 19.00 h, were ether anesthetized, a ventral laparotomy performed followed or not by the extirpation of the left or right adrenal. An untouched control group was included. The animals were sacrificed by decapitation one hour after surgery. Concentrations of corticosterone in serum were measured by Radio-Inmuno-Assay (RIA) using kits purchased from Siemens Healthcare Diagnostics Inc (Los Angeles, CA.) Results were expressed in ng/mL. Data on hormonal concentrations in serum were analized using multivariate analysis of variance (ANDEVA), followed by Tukey's test. A probability value of less than 5% was considered significant. Laparotomy performed at 7:00, 9:00, 13:00 or 17:00 h resulted in a significant increase in corticosterone concentrations, except in rats treated at 13:00 h of proestrus (P). The corticosterone levels in rats with left (Adx-L) or right adrenalectomy (Adx-R) were lower in comparison with animals with laparotomy, regardless of the time or the day in which performed the treatment. In comparison with corticosterone levels in control group, those animals with Adx-L treated at 7:00 h on D-2, 9:00 h on E, D-1 or P, or at 13:00 h on P, had higher levels of the hormone. A similar effect was observed in those rats with Adx-R treated at 9:00 h on E, D-1 or D-2 or at 13:00 h on P was higher than group control. Present results suggest that at 7:00, 9:00, 13:00 or 17:00 h the ability of the right and left adrenal to compensate the lack of one of them on the corticosterone serum levels is not different along the estrous cycle when the animals were studied. Supported by grant UNAM-DGAPA-PAPIIT IN215513-3.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 454.27/RR24

Topic: E.08. Biological Rhythms and Sleep

Support: NIH grant MH75968

Title: Glucocorticoid-dependent diurnal modulation of conditioned fear extinction and recall

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Abstract: Post-traumatic stress disorder (PTSD) is characterized by abnormal prefrontal cortex (PFC) functioning and abnormal circadian parameters (e.g. glucocorticoid [CORT] circulation). PTSD patients also exhibit persistence of a generalized fear response in the absence of immediate threat. This persistence of fear could at least in part be due to the inability to successfully extinguish conditioned fear and/or recall extinction. Exposure therapy is one of the most widely used treatments for PTSD, and the aforementioned extinction and memory deficits are exemplified by the low success rate of this treatment paradigm. Even when within session therapy is successful, fear responses tend to reappear outside the therapeutic context in a phenomenon known as fear renewal. CORT has been shown in animal and human studies to modulate both emotional memory and circadian rhythm integrity. PTSD patients frequently exhibit blunted circadian CORT release. We have previously shown that the molecular clock is modulated by CORT circulation patterns in the PFC, a brain area that is integral to proper conditioned fear extinction and recall. Because optimal PFC neural function may be reliant on molecular clock operation, we hypothesized that conditioned fear extinction learning and renewal may vary with time of day and the presence or absence of circadian CORT secretion. In these experiments we used a standard auditory conditioned fear protocol to train rats (n=6) to fear a tone (CS) that had been paired with a mild foot shock (US). 24 h later we presented rats with 15 trials of the tone alone to extinguish conditioned fear. 24 h later we tested rats for their ability to recall extinction. Either 72 hours (expt 1) or 24 hours (expt 2) later we put rats in a novel context and exposed them to 3 tones in order to measure fear renewal. Training and testing times were held constant and occurred either during the rat's active phase (Zeitgeber time [ZT] 16) or inactive phase (ZT 4). We found that conditioned fear extinction recall was enhanced and fear renewal was blunted in rats trained and tested at ZT16 compared to ZT4. These diurnal

effects on extinction recall and renewal were absent in adrenalectomized (ADX) rats, with level of responses at both times of day comparable to those of sham-ADX rats trained and tested at ZT4. We conclude that the recall of extinction as well as fear renewal are affected by both ZT and CORT status, such that optimal recall is reliant on training/testing that occurs during the active phase an in the presence of circulating CORT. This study holds implications for optimizing PTSD treatment by targeting both the circadian system (i.e. diurnal CORT) and persistent fear.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant AA017898

Title: Skimming the surface: Elucidating cellular mechanisms associated with tolerance to alcohol using the suprachiasmatic nucleus (SCN)

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Abstract: Alcohol abuse is linked to many disorders, including cancer, mood disorders, and sleep disturbances. The strong connection between sleep and circadian disruptions led us to investigate ethanol's effect on the circadian clock. Our research uses the SCN as a model system to investigate the progression of ethanol tolerance. We have shown that acute ethanol blocks photic phase shifts *in vivo* and glutamatergic phase shifts *in vitro* (Prosser et al, 2008). However, neural systems become tolerant to ethanol across acute, rapid and chronic timeframes. We have demonstrated that the SCN clock exhibits acute (within <30 min) tolerance to ethanol *in vitro* (Prosser and Glass, 2009), and rapid tolerance to ethanol both *in vivo* and *in vitro*, which develops 8-24 h after initial consumption (Lindsay et al, 2014). For rapid tolerance experiments C57BL/6J mice were given access to 15% ethanol/water solution during a single night. SCN brain slices were made the following morning and treated at either at zeitgeber time (ZT) 16

(where ZT 0 = lights-on and ZT 12 = lights off) or at ZT 23 with glutamate (1mM) +/- ethanol (20mM) for 10 min. The next day *in vitro* spontaneous SCN neuronal activity was monitored to determine the time of peak activity. Our results demonstrated that rapid tolerance occurs in the SCN, and this effect lasts between 48-96 hours. We have also investigated how ethanol tolerance and withdrawal alter NMDA receptor dynamics in the SCN. Our results indicate that total NMDA receptor NR2B subunit expression and NR2B phosphorylation at Tyr 1472 does not change during rapid tolerance to ethanol (Lindsay et al 2014). In contrast, we find that NR2B total expression increases during withdrawal from ethanol, and Tyr 1472 phosphorylation is increased during both chronic tolerance and ethanol withdrawal. Currently we are investigating potential changes in surface expression of NR2B using biotinylation, both across the circadian cycle as well as in response to ethanol consumption. For circadian expression, we investigated surface expression of NR2B at ZT6, ZT16, and ZT23. Our baseline experiment suggests that surface expression of NR2B is highest at ZT16 and lowest at ZT23. We have also determined NR2B surface expression at these time points after rapid tolerance. We find that overall surface expression of NR2B is greater after rapid tolerance relative to baseline, and is greatest at ZT16. Because the cellular mechanisms of tolerance to EtOH are not well known, understanding the dynamics of the NMDA receptor across different forms of EtOH tolerance may lead to novel pharmacological treatment for alcoholism. Support provided by the NIH: AA017898.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 454.29/RR26

Topic: E.08. Biological Rhythms and Sleep

Title: Caffeine potentiates circadian photic phase-resetting and delays light-entrained onset in mice

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Abstract: Caffeine is the most widely used psychoactive substance in the world, known for its ability to reduce sedation and increase alertness. However, long-term caffeine intake may adversely affect circadian rhythms, which has devastating impacts on health. In mammals,

circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus, a clock whose timing is entrained to the environment primarily by photic (light) input, mediated by glutamate release into the SCN from retinal afferents. Activation of presynaptic adenosine A1 receptors inhibits glutamate release in the SCN and blocks photic phase-resetting. As caffeine is an adenosine A1 receptor antagonist, we investigated its effect on photic phase-resetting and light-entrained activity rhythms in male C57BL/6J mice. To assess entrainment, a group of mice (n=18) was maintained in a 12 h light/12 h dark (LD) photocycle, receiving water alone for 14 days to assess baseline circadian entrainment and activity rhythms. Mice then received caffeine (1.0 mg/mL in water) for 14 days, during which caffeine intake and body weight was measured every 2-3 days. Mice consumed 102.40 ± 4.41 mg/kg/day caffeine. Caffeine delayed daily activity onset in LD from a baseline average of 26.04 ± 4.04 min after Zeitgeber Time 12 (ZT12; beginning of lights-off) to 101.50 ± 13.34 min after ZT12 (n=18, t=6.17, P<0.0001). Interestingly, caffeine reduced alpha (active-phase duration) from a baseline of 11.85 ± 0.14 h to 11.27 ± 0.26 h (n=18, t=2.58, P=0.0193). To assess photic phase-resetting, mice maintained in LD as above received water alone for 14 days, then caffeine (1.0 mg/mL in water) or water alone for 14 days, during which caffeine or water intake and body weight was measured as above. On day 15 after caffeine introduction, a 30 min light pulse was delivered at ZT14 (2 h after lights-off), after which mice were released into constant darkness to assess phase-delay responses. Water-drinkers (n=11) consumed 3.63 ± 0.42 mL water per day. Caffeine-drinkers (n=11) consumed 2.68 ± 0.16 mL of caffeine solution (103.97 ± 5.33 mg/kg/day caffeine; comparable to the mice used in the entrainment experiment). Caffeine exposure potentiated phase-delays in mice, with water-drinkers (n=11) delaying by 0.83 ± 0.05 h and caffeine-drinkers (n=11) delaying by 1.33 ± 0.15 h (t=3.25, P=0.004). These data show that caffeine potentiates circadian photic phase-resetting *in vivo* and delays entrainment to LD, supporting the hypothesis that caffeine may interfere with the ability of the SCN to entrain normally to light.

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Poster

454. Suprachiasmatic Nucleus and Circadian Rhythms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 454.30/RR27

Topic: E.08. Biological Rhythms and Sleep

Title: Pronounced impact of out of phase food intake on learning and memory

Authors: *D. H.-W. LOH[1], R. E. FLORES[1], D. TRUONG[1], S. A. JAMI[2], C. A. GHIANI[1], T. J. O'DELL[2], C. S. COLWELL[1]
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Abstract: The circadian system is a finely tuned network of central and peripheral oscillators headed by a master pacemaker, the suprachiasmatic nucleus (SCN). This network of oscillators governs daily rhythms in behavior and physiology, including cognition. Disruption of the circadian system by genetic mutations or environmental manipulations has severe consequences on cognition. In this study, we sought to determine the effects of chronic but stable misalignment of the circadian network by scheduling access to food at an inappropriate phase of the daily cycle. This manipulation alters the phase of many peripheral circadian oscillators without affecting the SCN. Mice were allotted a six hour window in which food was made available either during their active phase (aligned), or during their inactive phase (misaligned). We determined that misaligned feeding also altered the temporal pattern of gene expression of the hippocampus. Chronic misalignment of food access resulted in reduced performance on the novel object recognition test and had a severe impact on the recall of contextual fear conditioning, indicating deficits in hippocampal-dependent learning and memory. Critically, although the temporal pattern of sleep was altered, there was no difference in the amount of sleep between the aligned and misaligned groups, thus ruling out effects of sleep deprivation on memory. At the physiological level, misaligned feeding led to deficits in hippocampal long term potentiation, suggesting a role for the circadian oscillator in regulation of hippocampal function. Our findings suggest that circadian misalignment of the hippocampal oscillator has far-reaching effects on not only hippocampal physiology, but also on long term memory, and highlight the importance of circadian regulation on cognition.

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Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.01/RR28

Topic: A.10. Adolescent Development

Title: Structural connectivity with the prefrontal cortex predicts amygdala response during emotional face processing in children

Authors: T. NASH[1], T.-V. NGUYEN[2], N. TURNER[2], P. KOHN[2], K. ROE[2], M. GREGORY[2], S. KIPPENHAN[2], H. RAAB[2], D. BOYLE[2], S.-M. WEI[2], P. MARTINEZ[2], *J. B. CZARAPATA[1], P. SCHMIDT[2], K. BERMAN[2]
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Abstract: There is evidence that structural integrity of the uncinate fasciculus (UF), one of the major white matter tracts connecting limbic and prefrontal regions, predicts amygdala activation to fearful faces1. However, few studies have investigated the developmental trajectory of the amygdala's structural and functional connectivity. In both children and adults, we examined the relationship between structural connectivity of the UF and functional activation of the amygdala during viewing of aversive emotional faces. Diffusion and functional imaging data were acquired using a 3T MRI scanner in 29 children (mean age=11, range 8-13; 12 females) and in 25 adults (mean age=35, range 25-44; 13 females). Diffusion data were processed with TORTOISE2. Left and right UF regions of interest (ROIs) were created using the JHU White Matter Tractography Atlas3, and average fractional anisotropy (FA) values were extracted bilaterally for each participant. During scanning, participants viewed either aversive emotional faces or scrambled images. Anatomically-defined ROIs were created with the WFU PickAtlas for the anterior cingulate cortex (ACC) and for the left and right amygdala. Within the amygdala ROIs, average BOLD signal change (aversive vs. scrambled) was extracted for each participant. Standard methods were followed to test for a psychophysiological interaction between the amygdala and the ACC4. Subsequent analyses were performed to assess the relationship between UF FA and functional activation. We found bilateral activation of the amygdala during the task for both children and adults ($p<0.05$, FDR). There was no significant difference between groups in gender, functional connectivity, or amygdala activity. Adults tended to have higher UF FA than children ($p = .02$). In children, but not in adults, there was a positive association between UF FA and amygdala activation for the left ($r = .57$; $p = 0.001$) and right ($r = .58$; $p = 0.001$) hemispheres. This relationship was statistically stronger for children versus adults for the right hemisphere ($p = .02$), while there was a trend for the left ($p = .07$). Increased UF FA also predicted greater functional connectivity between the left amygdala and ACC ($r = .45$; $p = 0.02$) in children, but not in adults, with a trend toward a group difference ($p = 0.11$). In children, increased integrity of the UF predicted increased amygdala activity and functional connectivity with the ACC during viewing of aversive emotional faces, while this relationship was not present in adults. These results suggest that white matter integrity may impact the functioning of the fronto-amygdalar circuitry differently in children than in the fully developed adult brain.

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Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.02/RR29

Topic: A.10. Adolescent Development

Title: Morphometric comparisons of homosexual and heterosexual celebrity faces

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Abstract: Embryological development of the face and anterior brain occur in synchrony, are often influenced by common genes, and have the potential to be dramatically and characteristically altered by genetic abnormalities or environmental insults. To a statistically significant degree, observers examining photographs have successfully identified the sexual orientation of males and females after only a brief exposure. Examining only female homosexuals, photographs restricted to a window of face revealing only eyes and nose bridge are sufficient for guessing orientation beyond chance. In quantitative analyses, homosexuals have been found to have significantly less symmetrical faces. We found white individuals on the Web that were identifiable as having heterosexual (M = 35, F = 35) or homosexual (M = 35, F = 35) gender preferences and had photographs of the head directly facing the camera with the mouth closed and a neutral emotional expression. These faces were analyzed for 39 facial loci. The x-y coordinates of these loci were processed with a full Procrustes superimposition. These were then used to assess symmetry using deviations of loci when mirrored on the x axis. Distances were also calculated for a number of eye loci as well as facial structures known to be associated with variations in androgen exposure. ANOVA of facial symmetry revealed significant main effects for Sexual Orientation ($p = .001$), and Sex ($p = .001$), but no significant Orientation x Sex interaction ($p = .643$). Homosexual and Female faces were less symmetrical. When the analysis was restricted to only the eyes and nose bridge, there was a significant main effect for only Orientation ($p = .001$, homosexuals less symmetrical), but neither Sex ($p = .595$) nor Orientation by Sex interaction ($p = .599$). When looking at androgen-sensitive sites, the most striking results related to the width of the chin, which showed a significant main effect for Orientation ($p < .001$), but not Sex ($p = .070$), and a significant interaction ($p < .001$). Heterosexual males have wider chins, with homosexual males having chins narrower than females. The bridge of the nose showed a significant Orientation ($p < .001$), but not Sex ($p = .128$) effect, with a significant interaction ($p = .001$). Homosexual males, in particular, had wider nose bridges. Iris diameter showed a significant main effect for Orientation ($p = .001$) and Sex ($p < .001$), but not an

interaction (p = .675). Homosexuals and males have smaller iris diameters. These data reveal morphometric differences associated with sexual orientation that could be used when inferences are made about an unknown face and may provide information regarding variables affecting sexual orientation.

Disclosures: J. Cannon: None. **E. Akpan:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.03/RR30

Topic: F.01. Human Cognition and Behavior

Title: Memory for a panoramic visual environment shapes moment-to-moment scene representations

Authors: *C. E. ROBERTSON[1,2,3], K. HERMANN[3], D. KRAVITZ[4], N. KANWISHER[3,2,2]
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Abstract: As we navigate around our visual environment, our awareness of the place we are in seems to extend beyond the specific part of the environment that is currently in view to include a broader representation of the scene all around us. Here, we tested whether memory of a broad, panoramic representation of a novel space influences the ongoing representations of discrete views from within that panorama. Specifically, we tested whether discrete, nonoverlapping views of a scene become linked through a broader representation of their shared environment. Participants studied videos that each captured 100° of the 360° of a panoramic scene in the Beacon Hill neighborhood of Boston (Training Phase). Each video was displayed through a moving aperture that slid between the edge and center of the screen. On each trial, two videos were presented sequentially, one on each side of the screen (left/right). The two videos were either drawn from: 1) the same panoramic scene, with 20% of their content overlapping (Paired); 2) the same panoramic scene, but with no shared content (Unpaired); or 3) different panoramic scenes (Different). Participants subsequently performed two memory tests. During each trial of the memory tests, participants were shown two, non-overlapping still images, drawn from the studied panoramas. During the Explicit Test, participants judged whether the two stills depicted

the same location in Beacon Hill. During the Implicit Test, participants reported the spatiotopic position (right / left screen side) in which a still had appeared during training, after being primed with a brief (0.3 s) presentation of the other still. Preliminary results: Accuracy during the explicit test was higher if the two stills were drawn from the Paired rather than Unpaired conditions ($p<0.01$). During the implicit test, performance was above chance for all conditions, and participants were faster and more accurate to remember the spatiotopic position in which a discrete view was learned (left/right) if the two images (test/prime) were drawn from the Paired rather than the Unpaired condition (RT: $p<0.05$; Accuracy: $p<0.13$). This result indicates that forming a panoramic representation across two discrete views of a scene facilitates priming between these two views, beyond simple temporal contiguity during the training phase (which equally affected the Unpaired condition). These results suggest that a broad representation of a spatial environment influences the ongoing representation of discrete views from within that panorama, perhaps through "pattern completion", by which fragments of an experience can activate a population of neurons coding for an entire memory or spatial environment.

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Poster

455. Face and Scene Perception

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Topic: F.01. Human Cognition and Behavior

Title: Turning the body inside-out: Insular cortex activity reflects interoceptive and exteroceptive integration - A 7T study

Authors: *M. BLEFARI[1], R. MARTUZZI[1,2], A. SERINO[1,2], O. BLANKE[1,2,3]
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Abstract: Bodily self-consciousness (BSC), the experience of self as being located within a body perceived as one's own, depends on multisensory integration of body signals. Manipulations based on multisensory stimulation can alter BSC, as in the rubber hand illusion or full body illusions. Thereby viewing a tactile stimulation of an artificial body part, while synchronously feeling touch on one's own body, induces a sense of ownership for the artificial

body and a shift in the perceived location of one's own self towards the artificial body. Traditionally, research on multisensory integration focused on exteroceptive signals, with interoceptive signals being explored only recently. Our group has shown that viewing a virtual body periodically illuminated in synchrony with participant's heartbeat induced illusory ownership of the virtual body. However, the neural mechanisms of cardio-visual integration are unknown. Here, we investigate cardio-visual signals' integration in the brain and whether their neural activation overlap with activations during a heartbeat awareness task. Sixteen healthy participants took part in our 7T MRI experiment. Heart rate was monitored using pulse oximetry. The onset of each pulse triggered the flashing outline superimposed on a virtual body (or on inanimate object, serving as a control condition), shown to the participants. The experimental protocol was divided in two consecutive steps. *Cardio-Visual Stimulations (CVS)*. Subjects were required to look at either a virtual body or at an inanimate object, whilst an outline contour flashed synchronously or asynchronously with the subject's heartbeat. *Heartbeat Awareness Task (HAT).* Subjects performed randomized trials of a heart or a color task. In the heart task, they were required to answer whether a rectangular stimulus flashing in front of them was synchronized to their heartbeat, while in the color task whether the rectangular stimulus changed its color. CVS significantly activated clusters in the right Frontal Inferior Operculum (FIO), left Insula (Ins), and right Supramarginal Gyrus (SMG). The HAT was associated with activation in bilateral insular cortex and right SMG. A region of interest analysis on overlapping regions between CVS and HAT revealed a stroking and body-selective activation in the right FIO and a synchronous stimulation activation independent of whether a body or control object was seen in the SMG. We show that interoceptive and exteroceptive signals integration (that impact bodily self consciousness) is processed in the insular cortex. The relevance of these data for multisensory integration and neurobiological models of self-consciousness is discussed.

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Poster

455. Face and Scene Perception

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Topic: F.01. Human Cognition and Behavior

Support: R01DA015179

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T32DA024635

Title: Dopamine D2-type receptor availability contributes to neural activation in the human amygdala during performance of a facial affect-matching task

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Abstract: Background: The amygdala plays an important role in arousal and affective experience, and evidence points to dopamine signaling as a potential contributor to such functions of the amygdala. Pharmacological and electrophysiological studies of rodent models have indicated a role for the dopamine D2-type receptor, but a study using positron emission tomography (PET) and fMRI showed an involvement of D1-, but not D2-type dopamine receptors to amygdala activity related to viewing images of faces with both neutral and negative emotional expressions and categorizing them by gender. Here, we tested further for D2-type dopamine receptor involvement in amygdala activation using an fMRI contrast that is specifically related to processing emotional facial expressions. Method: Fifteen healthy volunteers [8 men, 7 women; 31.7 ± 8.01 (mean ± SD) years of age] participated. PET with [18F]fallypride, a radiotracer with high affinity for dopamine D2-type receptors, was used to measure receptor availability, indexed by binding potential (BPND). The Facial Affect-Matching Task was used with a block design during fMRI to elicit task-related brain activity in the amygdala. In the "match affect" condition, a reference face, showing fear or anger, was presented in the top-middle portion of the screen. In the sensorimotor control condition (the "match shape" condition), an irregularly shaped black blob was presented in the top-middle portion of the screen. In each condition, participants were instructed to select which of the two faces or shapes presented at the bottom of the screen matched the reference face (by affect) or shape at the top. The contrast, "match faces > match shapes," was used to assess amygdala activation. Multiple linear regression analysis was performed, with fMRI parameter estimates set as dependent variables, and age, gender and D2-type BPND set as the independent variable. Result: The overall model for the left amygdala showed a significant result ($p = 0.043$, adjusted $R2 = 0.375$), with BPND being the only significant predictor of the fMRI response ($\beta = 0.640$, $p = 0.029$). The overall model for the right amygdala did not yield a significant result ($p = 0.493$, adjusted $R2 = -0.032$). Conclusion: D2-type dopamine receptor availability, a marker for dopamine signaling, contributes to amygdala activation during facial-affect processing in the left amygdala. Discrepancies between this finding and a previous one may reflect differences in the fMRI task used to assess amygdala activation. Lateralization in this result is consistent with prior studies that revealed robust activation and dopamine release during emotional tasks in left amygdala.

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Poster

455. Face and Scene Perception

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Title: Neural correlates of body representation impairments after stroke

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Abstract: A representation of our body is necessary to perceive, locate or identify body parts. Body representation can be disrupted after brain damage such as a stroke, resulting in various deficits such as finger agnosia, autotopagnosia and left-right disorientation. Previous neuroimaging studies with healthy participants have suggested a central role for the posterior parietal cortex, the secondary somatosensory cortex and the insular cortex. So far few group studies have investigated the neurological basis of body representation impairments. In this study, we conducted a neuropsychological assessment in the subacute phase after an ischemic stroke in 50 patients. This assessment included tasks aimed at body representation impairments, e.g. finger agnosia, tactile localisation and left-right disorientation. Patients that show selective deficits in their body representation were selected and compared with stroke patients with no or other cognitive deficits. Voxel-based lesion-symptom mapping was applied to reveal neural correlates that were associated with body representation impairments. Preliminary analyses reveal that differential lesion locations are associated with different impaired body representation components. These findings will be discussed in relation to current models of the neural basis of body representation.

Disclosures: **H.E. van Stralen:** None. **J.M. Biesbroek:** None. **D. Sluiter:** None. **H.M.A. van Gemert:** None. **L.J. Kappelle:** None. **M.J.E. van Zandvoort:** None. **G.J. Biessels:** None. **C. Dijkerman:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.07/RR34

Topic: F.01. Human Cognition and Behavior

Support: NEI R01EY024161

Title: Behavioral evidence that ultra-fast face detection relies on early, non-holistic face representations

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Abstract: Prior studies have indicated that the human visual system can perform rapid object detection based on a single pass through the visual hierarchy, in about 200ms. However, this "Standard Model" was recently challenged by demonstrations that reliable saccades to images containing faces were initiated as early as 120-130 ms after image onset (Crouzet et al. 2010, 2012). The short latency of these saccades suggest that instead of the classic hierarchical model, in which objects can only be coded at the very top of the system, "objects" can be detected by neurons located in early areas, especially when those objects are biologically important and their detection requires receptive fields with resolutions only found in lower visual areas. We tested the hypothesis that, given their putative early position in the visual hierarchy, such low-level face detectors would be predicted to show less object-selectivity than high-level face detectors at the top of the ventral stream. We asked subjects (n=8) to saccade to faces displayed in natural scenes together with a distractor, each 2 deg in size and presented at 7 deg eccentricity. There were 4 different distractor conditions, presented in random order: house, phase-scrambled face, inverted face, and upright face with face parts moved to non-standard configurations ("face-part" condition). Selective saccades toward faces were triggered very rapidly (mean minimal reaction time for selective saccades from 137 to 152 ms across conditions). Interestingly, in face-part trials, the first saccade was as likely to be directed to distractors as to the face target ($p > 0.2$,ttest), while it was more often directed to the face target in the 3 other conditions ($p<0.001$).

In addition, a high number of saccades toward distractors in the inverted face condition suggested that they also interfered with the execution of the task, but to a lesser extent than face-parts distractors. Moreover, there was evidence that the face-likeness of the distractor affected the accuracy of saccades to face targets, with target spatial localization error being significantly higher in the face-part and inverted-face conditions than in the house and scrambled conditions. Our data suggest that ultra-fast saccades to faces are driven by spatially localized face-selective but non-holistic neuronal representations, putatively in low-level visual areas that show the required combination of small receptive field sizes and short activation latency to account for the behavioral performance. Thus, our data strengthen the hypothesis that early visual areas can be recruited in complex object detection tasks to allow ultra-rapid and spatially accurate object detection.

Disclosures: F. Campana: None. **J.J. Martin:** None. **X. Jiang:** None. **S.J. Thorpe:** None. **M. Riesenhuber:** None.

Poster

455. Face and Scene Perception

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Program#/Poster#: 455.08/RR35

Topic: F.01. Human Cognition and Behavior

Title: Impact of task context on the cortical representations of real-world scenes

Authors: *M. KING[1], A. HAREL[1], D. KRAVITZ[2], C. BAKER[1]
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Abstract: Humans are extremely adept at recognizing complex real-world scenes, an ability supported by scene-selective regions in occipito-temporal cortex including the Parahippocampal Place Area (PPA) on the ventral surface, and Transverse Occipital Sulcus (TOS) on the lateral surface. Previous research has shown that neural response patterns in these regions primarily represent spatial properties (open/closed) and not semantic content (manmade/natural)(Kravitz et al., 2012). However, these results were obtained while participants performed an orthogonal fixation task and the extent to which these representations might be modulated by top-down, observer-based factors (e.g. task context) remains unclear. To determine how top-down factors impact scene representations, we performed an event-related functional magnetic resonance imaging (fMRI) experiment manipulating both visual scene properties and task context. Participants viewed a diverse set of real-world scenes, spanning three scene dimensions (layout:

open/closed, content: manmade/natural, distance: near/far). Critically, participants were asked to perform two different tasks in which specific stimulus dimensions were either relevant or irrelevant: a layout task ("Is the scene open or closed?") or a semantic content task ("Is the scene manmade or natural?"). Participants performed the two tasks while viewing the exact same scene images, ruling out any potential stimulus effects. We hypothesized that representation of each dimension would be enhanced when that dimension was task relevant. Surprisingly, we found that scene representations in PPA and TOS are largely independent of task context. Scene representations were primarily dominated by layout, even when that dimension was not relevant for the task at hand (i.e. during the semantic content task), with little change to the representation of semantic content. Instead, task context modulated the representation of individual scenes, reflecting an interaction between those scene dimensions conveying spatial properties of the scene (near/far, open/closed) and the task. Together, these findings suggest that the global structure of scene representations in occipito-temporal cortex is largely unaffected by top-down processes, although the responses to individual scenes are task-dependent.

Disclosures: **M. King:** None. **A. Harel:** None. **D. Kravitz:** None. **C. Baker:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1 EY012440

Title: Are individual differences in perception of bistable figures related to visual imagery preference?

Authors: L. TAYLOR, *S. A. LACEY, Y. DOAN, K. SATHIAN
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Abstract: Bistable figures are those for which perception alternates between two interpretations, despite the absence of any change in the perceptual information available in the figure itself: classic examples are the 'duck/rabbit' and the Necker cube figures (Attneave, Sci Am, 225:62-71, 1971). Individual differences in the ability to see figure reversals (i.e., the alternating interpretations of the ambiguous figure) have been related to creativity (Wiseman et al., Brit J Psychol, 102:615-622, 2011; Doherty & Mair, Perception, 41:1262-1266, 2012); however,

science students see more reversals than arts students (Doherty & Mair, ibid.). Individual differences in visual imagery have been characterized in terms of preferences for object or spatial imagery: whereas object imagers experience highly depictive images, spatial imagers are better at image transformation (Kozhevnikov et al., Mem Cognition, 33:710-726, 2005). Moreover, spatial imagers are over-represented in the sciences and object imagers are over-represented in the arts (Blazhenkova & Kozhevnikov, Appl Cognitive Psych, 23:638-663, 2009). Hypothesizing that alternating between bistable percepts is analogous to image transformation, we predicted that spatial imagers would see alternative interpretations faster and more often than object imagers. Participants viewed four natural (e.g., duck/rabbit) and four geometric (e.g., Necker cube) bistable figures, each for two minutes. We recorded the time taken to see the first figure reversal and the number of subsequent reversals. Participants also completed the Object-Spatial Imagery and Verbal Questionnaire (OSIVQ; Blazhenkova & Kozhevnikov, ibid.) together with visual (Pattern Meanings) and verbal (Alternate Uses) creativity tests (Wallach & Kogan, 'Modes Of Thinking In Young Children', 1965). Response times (RTs) and the number of figure reversals observed were significantly correlated with imagery preference, such that RTs decreased as preference for spatial imagery increased and the number of reversals decreased as preference for object imagery increased. Creativity measures were not correlated with RTs, the number of reversals, or with imagery preference. Our results suggest that spatial imagers, being more accustomed to image transformations, have an advantage over object imagers in the ease with which they detect the different interpretations of bistable figures. Since bistable figure perception does not involve either imagery or a memory load, our results also suggest that imagery preferences may be related to differing perceptual strategies, perhaps affecting how the visual scene is parsed.

Disclosures: L. Taylor: None. **S.A. Lacey:** None. **Y. Doan:** None. **K. Sathian:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.10/RR37

Topic: F.01. Human Cognition and Behavior

Title: The nature of texture representation in the PPA

Authors: *J. PARK, S. PARK
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Abstract: The parahippocampal place area (PPA) has been traditionally viewed as an area encoding the spatial layout of a scene (Epstein & Kanwisher, 1998). In contrast, lateral occipital area (LO) has been suggested to process non-spatial information such as objects or textures (Kravitz et al., 2011; Park et al., 2011). Recently, there has been a series of studies challenging this traditional view (Cant & Goodale, 2011; Kornblith et al., 2013). For example, a recent study suggests that the PPA is sensitive to changes in texture ensemble (Cant & Xu, 2012) and change of ratio between textures (Cant & Xu, 2012 VSS). These series of evidence suggest an involvement of the PPA in representing texture information, but less is known about the nature of texture representation in the PPA. In this study, we test two hypotheses about the nature of texture representation in the PPA: First, the texture may provide information about the "identity" of a scene. Even with the same spatial structure, the identity of place could change radically depending on kinds of texture (e.g., different wall papers across rooms) or specific positions of textures within a space. Specifically, a scene can have the same 'mean' texture, but have different 'identity' depending on positions of textures within a scene. If the PPA encodes the identity of a scene from texture, it will be sensitive to specific positions of each texture even when the mean texture is equated. Second hypothesis is that the PPA represents textures as is, showing no sensitivity to positions of specific texture or to the identity of a scene. In this case, PPA will be sensitive to kinds of textures and the ratio of each texture, but not to their individual positions. In an fMRI scanner, we presented images of synthetic rooms composed of floor and ceiling (FC), left and right walls (LR), and a middle wall. To equate mean texture in a scene, the amounts of areas of FC and LR are equated. FC always has the same texture, and the LR always has the same but different texture from that of FC. Two different kinds of texture sets per scene were used. In a critical 'swap' condition, the texture of FC and LR are swapped. This swapped image has the same kinds, ratio and mean of textures, but different scene 'identity'. In 'rotation' condition, a viewpoint of the image is rotated, which has the same kinds of texture, same scene identity, but different ratio of the textures due to the changed amount of walls shown. By using MVPA correlation method, we observe the pattern of neural similarity matrix in comparisons to our hypothetical representational matrices. The results of this study will provide valuable information to re-evaluate the role of PPA in scene perception.

Disclosures: J. Park: None. **S. Park:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.11/RR38

Topic: F.01. Human Cognition and Behavior

Title: Eye-tracking parameters on visual scenes with emotional content

Authors: *W. C. DE SOUZA, A. M. MELCHIADES, G. A. JANCZURA, M. A. G. FEITOSA
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Abstract: Eye movements have been described as indicative of cognitive processes that occur at the same time its own existence. Thus, they can offer valuable insight into how the brain organizes the constant flood of information nearby. The objective of this study was to investigate possible relationships between eye movements during visual tracking of stimuli with emotional contents. So, the variables pupil size, fixation and saccade duration times and number of fixations and saccades were measured, aiming to assess the impact of the independent variables emotion, type of task and sex. The study consisted of a sample of 55 participants between 18 and 30 years, 52.9% men and 90.9% with incomplete higher education, organized into eight experimental groups according to the profile of emotional stimuli. The experimental task consisted on the presentation of a series of images of complex visual scenes, about four major themes (people, human actions, animals and environment), while participants watched them sometimes freely, sometimes under the task of judging their loads of valence and alert. The study results suggest that pupillary sizes were influenced by the variables emotion and type of task, as well as the correlation between these two variables. The time spent in saccades was influenced by the type of task, being higher in free search. Finally, fixation and saccade time in directed search were influenced by gender, whereas women tend to perform more fixations than men during directed search. The data resulting from this work suggest the influence of emotion, type of task and gender of participant in eye movements performed by a scene observation. As initially hypothesized, not only semantic information but also emotional aspects appear to be involved in the execution of eye movements in front of a complex visual scene.

Disclosures: W.C. De Souza: None. **A.M. Melchiades:** None. **G.A. Janczura:** None. **M.A.G. Feitosa:** None.

Poster

455. Face and Scene Perception

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.12/RR39

Topic: F.01. Human Cognition and Behavior

Title: Decoding face retrieval and reconstructing face perception from activity patterns in posterior parietal cortex

Authors: *H. LEE[1], A. S. COWEN[2], B. A. KUHL[1,3]
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Abstract: Posterior parietal cortex (PPC) is thought to support both working memory maintenance and long-term memory retrieval. However, whether PPC actively represents the details of remembered content--as opposed to supporting content-general memory operations--remains a point of debate. In the current study, we applied high-resolution fMRI pattern analysis methods to test whether perceptual details of a stimulus are represented in PPC during encoding and retrieval. In contrast to prior studies that have tested for sensitivity to broad visual categories (e.g., faces vs. scenes), here we tested for sensitivity to individual face images. In Experiment 1, we employed a retro-cue working memory paradigm (Harrison and Tong, 2009) in which two faces were presented in rapid succession followed by a cue to retrieve/maintain one of the two faces. A total of 32 unique faces were used. Neural pattern similarity was computed, across trials, between cued faces using delay period activity. We found that pattern similarity in PPC during the delay was greater across trials corresponding to the same cued face relative to trials with different cued faces. Thus, the individual face image being remembered could be decoded from activity patterns in PPC. To more directly test whether PPC represents visual details of face images, we tested (in Experiment 2) whether activity patterns in PPC during face perception/encoding could be used to reconstruct individual face images using a recently described method (Cowen, Chun, and Kuhl, 2014). In this experiment, participants viewed hundreds of faces along with occasional scene images (included in order to localize face-selective brain regions). The task was continuous recognition, where participants judged whether each image was novel ('new') or repeated within a block ('old'). We generated face reconstructions by first mapping information in the face images to patterns of fMRI activity (using a set of 'training faces') and then predicting face information from activity patterns (using a distinct set of 'test faces'). The predicted face information was then used to generate a reconstruction which could be compared to the face image that was actually viewed (as well as to other face images). Reconstructions generated from PPC exhibited above-chance similarity to the actually viewed face, indicating that visual information about individual face images is reflected in PPC activity patterns. Together, these findings support the idea that PPC actively represents exemplar-level details of stimuli that are encoded into and retrieved from memory.

Disclosures: H. Lee: None. **A.S. Cowen:** None. **B.A. Kuhl:** None.

Poster

455. Face and Scene Perception

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 455.13/RR40

Topic: F.01. Human Cognition and Behavior

Support: Wofford College

Title: Cortisol variation at retrieval differentially impacts memory for objects and backgrounds in emotional scenes

Authors: *K. R. MICKLEY STEINMETZ[1], B. E. FLEMMING[2], A. M. HENSON[2]
[1]Psychology, [2]Wofford Col., Spartanburg, SC

Abstract: High arousal states engage the hypothalamic-pituitary-adrenal (HPA) axis, which may lead to a biased memory towards emotional aspects of scenes at the expense of memory for backgrounds. Stress-induced changes measured by variation in the stress hormone, cortisol, were documented. Participants encoded scenes that included either a high-arousal, moderate-arousal, or neutral object placed on a neutral background. After either a stressor or a control condition, recognition memory was assessed for objects separately from the backgrounds. Elevations in cortisol at retrieval were associated with impaired memory for objects in the control group, but improved memory for backgrounds in the stress group. In addition, greater cortisol reactivity in response to a stressor was associated with more false alarms, specifically for negative information. These findings suggest that levels of cortisol, in reaction to stress, differentially impact memory for objects and backgrounds.

Disclosures: K.R. Mickley Steinmetz: None. **B.E. Flemming:** None. **A.M. Henson:** None.

Poster

455. Face and Scene Perception

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Program#/Poster#: 455.14/RR41

Topic: D.09. Tactile/Somatosensory

Support: NSF Grant to LTLikova

Title: Commonalities between visual art and music in insular cortex revealed by hemispheric asymmetry for visual but not for blind drawing

Authors: *L. T. LIKOVA, S. NICHOLAS
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Abstract: Striking unilateral activation has recently been discovered in left insular cortex (IC) for listening to melodies previously practiced on a musical instrument, although,the underlying mechanism is not yet known. Does this deep post-training asymmetry reflect a form of higher order, art-related cognition? Or a lateralized motor learning re-activation? We addressed these questions in a multimodal drawing-training paradigm. Methods: Brain activity was compared under both visual and tactile conditions by running fMRI scans before and after training in drawing for both sighted and blindfolded participants. The effect of training was probed in four experimental tasks: i) passive image exploration (by viewing or touching), ii) active reproduction of the explored images by drawing from memory (visual or tactile), iii) active reproduction by observational drawing (i.e., drawing images that are present), and iv) non-image drawing (scribble) as a motor control task. Results/Conclusions: In the visual conditions, prior to training, a left IC region was engaged during image exploration and observational drawing, but not during memory drawing or scribble. Post training, however, both forms of drawing - drawing from memory and from observation - generated a highly significant left IC activation, while the exploration activation was reduced. The motor-control task, scribble, did not activate left IC, excluding the motor re-activation hypothesis. Surprisingly, in the blindfolded condition, there was no activation in any task either pre- or post-training, implying that left IC role was restricted to the visual modality only. In contrast to the left IC, the right IC - that was recently attributed a major role in switching activation/deactivation between brain networks - was massively deactivated under both visual and non-visual conditions. These results will be interpreted in a novel framework including presence-of-self, agency and embodied cognition in art, rather than simple re-activation of hand-representation.

Disclosures: L.T. Likova: None. **S. Nicholas:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: Air Force Office of Sponsored Research grant FA9550-10-1-0385 to R.P.

Title: Comparison of cognitive training vs transcranial Direct Current Stimulation on performance of a "Cyber Defense" multi-task

Authors: E. CLAYTON[1], D. CISLER[1], R. MCKINLEY[2], M. BIKSON[3], *P. M. GREENWOOD[1], R. PARASURAMAN[1]
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Abstract: Cyber defense operations are aimed at defending computer networks - critically important for protecting military, government, and business assets from theft and disruption. Cyber operators detect and investigate anomalies missed by automatic software defenses. Simultaneous cognitive demands on such operators involve vigilance, working memory, and visuospatial attention. It is important to develop ways to optimize performance on such complex multi-tasks. Cognitive Load Theory (Wickens, 2012) argues that reducing task load during training makes more resources available for learning, e.g., by part-task training on subtasks. Another approach is non-invasive transcranial Direct Current Stimulation (tDCS) aimed at mediating brain regions of component subtasks. We hypothesized that a cognitive training intervention would be more effective in enhancing the targeted subtask than tDCS due to greater specificity. To test this hypothesis, we used the multi-task Cyber Defense Task (CDT) developed by University of Dayton Research Institute in consultation with the Air Force to simulate cyber defense operations. The task requires simultaneous monitoring of a (a) Graphical subtask (detection of traffic volume over a network) and (b) Textual subtask (memory for suspicious internet protocol address and port combinations). Young participants were randomly assigned to: (a) tDCS over left prefrontal cortex (F3); (b) tDCS over right parietal cortex (P4); (c) working memory (n-back) training; (d) visuospatial attention (flanker) training; (e) sham training/sham tDCS. All participants were administered CDT for a 10 minute baseline, 30 minutes of either training, 2.0 mA tDCS, or sham training/sham (.1 mA) tDCS, then 20 additional minutes of the CDT. We submitted nonparametric signal detection theory metrics for Graphical and Textual task performance to a MANOVA, using baseline performance as covariates and intervention group as the between factor. Preliminary results showed a difference between the groups (Wilks' lambda = .776, F=1.96, p=.057), with a significant univariate F (p=.023) for the Graphical task. Thus, the best performance was by the prefrontal stimulation group and the worst performance by the working memory training group. The Textual task did not show differential effects. Contrary to our hypothesis, tDCS was superior to cognitive training for improving signal detection in this simulated cyber defense setting. Further, working memory training impaired performance on the visuospatial attention subtask. Overall, tDCS was beneficial for multi-task performance while part-task training was not.

Disclosures: **E. Clayton:** None. **D. CIsler:** None. **R. McKinley:** None. **M. BIkson:** None. **R. Parasuraman:** None. **P.M. Greenwood:** None.

Poster

456. Direct Current Stimulation

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Topic: F.01. Human Cognition and Behavior

Support: Air Force Office of Sponsored Research grant FA9550-10-1-0385

Title: Intensive working memory training transfers to everyday functioning and alters connectivity between the dorsal and ventral attention networks

Authors: ***D. CISLER**, M. STRENZIOK, R. PARASURAMAN, P. M. GREENWOOD
George Mason Univ., Fairfax, VA

Abstract: A common aim of cognitive training is far transfer to general cognitive ability. Although working memory (WM) training has shown the strongest evidence to date of far transfer to fluid ability (e.g., Jaeggi et al., 2008), the literature is inconsistent. Based on previous evidence that successful cognitive training modulated connectivity between nodes of the dorsal (Takeuchi et al., 2010; Strenziok et al., 2014), and ventral (Strenziok et al., 2014) attention networks, even after only days of training (Lewis et al., 2009), we hypothesized that cognitive training would modulate functional connectivity in the dorsal and ventral attention networks early in training, whether or not far transfer was seen. We tested this with adaptive WM training due to previous evidence that it induced far transfer to fluid ability. Young adults were pre-tested and pre-scanned before being randomly assigned to adaptive WM training during either 2 mA or .1 mA (sham) tDCS. The supervised training was 1.5 hours a day (with tDCS or sham for 30 min of that time) for 4 consecutive days on adaptive WM memory tasks (n-back, spatial WM), followed by post-testing and post-scanning. Pre- and post-testing included: spatial working memory, visuospatial attention, WAIS Letter-Number Sequencing, WAIS Matrix Reasoning, Wechsler Memory Scale, Logical Memory Immediate and Delayed, and the Everyday Problems Test (Willis & Marsiske, 1993). Functional connectivity (fcMRI) images were acquired at rest with participants' eyes open in axial plane with a single-shot echo-planar sequence sensitive to change in the BOLD response (TR = 2500 ms, TE = 30 ms, 42 slices, 3mm3 resolution, flip angle 70°). A MANOVA on the behavioral data revealed effects of tDCS on transfer of training (Wilks' lambda, p=.048). Time to complete the Everyday Problems Test benefited from WM

training (p=.037). These findings of far transfer from WM training to the Everyday Problems Test are not consistent with the lack of transfer to Matrix Reasoning. Analysis of the fcMRI data involved a whole-brain seed-based correlation analysis in the dorsal attention network (superior parietal cortex, SPC), using a 2 mm spherical region previously reported to change following sensory training (Lewis et al., 2009; Strenziok et al., 2014). Preliminary functional connectivity analyses demonstrated a Group x Time interaction ($p < .05$) in the lateral occipital lobe indicating a larger decrease in connectivity in the sham group, compared to the tDCS group, from pre-to post training. This extends our previous finding of perceptual training-related functional connectivity change in older adults (Strenziok et al., 2014).

Disclosures: D. Cisler: None. **M. Strenziok:** None. **R. Parasuraman:** None. **P.M. Greenwood:** None.

Poster

456. Direct Current Stimulation

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Topic: F.01. Human Cognition and Behavior

Support: Air Force Office of Scientific Research Grant FA9550-10-1-0385

Title: Transcranial direct current stimulation differentially influences implicit and explicit memory in a multi-task

Authors: *M. R. SCHELDRUP[1], J. VANCE[2], R. MCKINLEY[3], M. BIKSON[4], R. PARASURAMAN[2], P. GREENWOOD[2]
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Abstract: Many cognitive multi-tasks are of high importance to society (e.g., air traffic control), yet training on such tasks can take weeks, months, or years. Non-invasive brain stimulation, such as transcranial direct current stimulation (tDCS), has promise as a method to facilitate such training. However, most previous tDCS studies measured effects only on one targeted cognitive ability, without evaluating effects -- including costs -- on other abilities. We hypothesized that explicit learning would benefit when the anode was over right dorsolateral prefrontal cortex (dlPFC, F4) while implicit learning would benefit when the cathode was over right dlPFC (to left arm), based on the assumption that suppression of explicit learning enhances implicit learning.

We tested this in the dual-task Warship Commander (DARPA Augmented Cognition Research Group), consisting of two concurrent tasks: (a) Airspace Monitoring which requires implicit learning of the complex rules by which a naval commander defends a convoy; (b) Ship Status which requires explicit learning of updates about systems throughout the ship. Participants performed the dual task for 20 min after random assignment to 2 mA tDCS over a) F4 anode b) F4 cathode or c) sham (.1 mA) on the first, training day. To assess retention, participants returned the next day for another 20 min block without tDCS. We predicted different effects on Training and Retention. Training: On Airspace Monitoring, there was a significant effect of Group on total number of buttons pressed during performance (p=.047), with post-hoc comparisons indicating that the F4 anode group reduced button presses compared to sham (p=.052). Number of incorrect button presses varied marginally by Group (p=.063). On Ship Status, the groups differed (p=.004), with the F4 anode group significantly slower to respond to queries compared to both sham (p=.007) and F4 cathode groups (p=.024). Retention: On Airspace Monitoring, number of buttons pressed was affected by stimulation group (p=.010), with the F4 anode group making fewer button presses compared to the sham group (p=.009). On Ship Status, the groups differed (Group, p=.017), with the F4 anode group responding more slowly to queries compared to sham (p=.025). Overall, these results show that the benefits of the F4 anode/left shoulder cathode montage on the implicit task may have been at the expense of the explicit task.

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Poster

456. Direct Current Stimulation

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Program#/Poster#: 456.04/RR45

Topic: F.01. Human Cognition and Behavior

Title: Augmenting mirror visual feedback by transcranial direct current stimulation

Authors: ***P. RAGERT**[1], E. VON REIN[2], M. HOFF[2], E. KAMINSKI[2], B. SEHM[2], C. J. STEELE[2], A. VILLRINGER[2]
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Abstract: Mirror visual feedback (MVF) has been shown to improve motor performance of the untrained hand. It has been suggested that plasticity in the primary motor cortex (M1)

representing the untrained hand is an essential component of MVF-induced performance improvements. Here we thought to determine if MVF-induced performance improvements of the left hand can be augmented by up-regulating the excitation of right M1 by means of anodal transcranial direct current stimulation (a-tDCS). So far, we enrolled 20 healthy right-handed participants (mean age 25 years) in a randomized, sham-controlled, double-blinded parallel design. All participants performed initially a ball rotation task with the left hand for 1 minute. The aim of the task was to rotate two balls in the left hand as fast as possible in counter clockwise direction. Subsequently, group 1 (a-tDCS) performed the ball rotation task with the right hand but in that case in clockwise direction 10 times for 1 minute with 1 minute rest blocks in between. Here tDCS was applied concurrently with the task for a total duration of 20 minutes (1mA intensity, anode over right M1, cathode over the contralateral orbit). Group 2 performed the same task but without tDCS (sham tDCS, s-tDCS). During the ball rotation with the right hand, all participants were asked to observe the respective movements of the hand in a mirror (MVF). After the training period with the right hand, all participants performed the ball rotation with the left hand again (counter clockwise orientation). While initial baseline performance of the left hand was not different between groups (T-Test: p=0.9), the a-tDCS group exhibited significant stronger MVF-induced performance improvements as compared to sham (TIME X GROUP interaction: F(1,18)=10.778; p=0.004). Based on the results, we provide novel evidence that up-regulating activity in the right M1 by means of a-tDCS is capable of augmenting MVF-induced performance improvements in young normal volunteers. Our findings further highlights the importance of plasticity in M1 contralateral to the untrained hand, a phenomenon that might be useful in translational research approaches that aim to use MVF as an adjuvant strategy in neurorehabilitation.

Disclosures: **P. Ragert:** None. **E. von Rein:** None. **M. Hoff:** None. **E. Kaminski:** None. **B. Sehm:** None. **C.J. Steele:** None. **A. Villringer:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 456.05/RR46

Topic: F.01. Human Cognition and Behavior

Title: Facilitating mirror visual feedback in the elderly by transcranial direct current stimulation

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Abstract: Introduction Healthy aging is typically accompanied by a progressive decline in cognitive, sensory and motor function, influencing activities of daily living. However, several previous studies indicated that age-related behavioral alterations are not irreversible. There is evidence from young normal volunteers that mirror visual feedback (MVF) during a ball rotation task improves performance of the trained and untrained hand. The question remains whether MVF also improves performance in the elderly. Non-invasive brain stimulation has previously been used as a tool to restore/enhance skill acquisition and motor performance in old adults by up-regulating brain activation. Here we thought to determine if transcranial direct current stimulation, a form of non-invasive brain stimulation, can be used to augment MVF-induced performance improvements of the untrained hand in the elderly. Method Twenty-four healthy elderly participants were tested in a randomized double-blind, sham controlled experimental design. Participants first performed a complex ball rotation task with the left hand (counterclockwise) for 60 s. Subsequently the task was performed with the right hand (clockwise) 10 times (60 s each) while MVF was provided. Afterwards, the left hand was tested again. Anodal tDCS (a-tDCS) was applied to the right M1 representing the (stationary) left hand, during the performance of the right hand concurrent with MVF. One group (n=12, 6 men, 66.17±5.79 years) received 20 minutes of a-tDCS (anode over right M1, cathode over frontal orbit), while the other group received sham stimulation (s-tDCS, n=12, 7 men, 66.5±5.00 years). Results Both groups showed a comparable initial performance of the left hand (a-tDCS: 35.92 ± 3.84 ball rotations/ min; s-tDCS: 31.17 ± 3.04 ball rotations/ min, t-test $t(22)=0.647$ $p=0.524$). Overall, MVF resulted in a significant performance improvement of the left untrained hand (ANOVA-RM with factor TIME $F(1,22)=28.962$ $p=0.000$). However, the a-tDCS group showed superior performance improvements as compared to sham (TIME x GROUP interaction $F(1,22)=5.244$ $p=0.032$), with an average gain of 11.17 ± 1.85 ball rotations/ min in the a-tDCS group, compared to 4.50 ± 2.25 ball rotations/ min in the s-tDCS group. Discussion We provide novel evidence that the application of a-tDCS over M1 of the stationary hand can further enhance MVF-induced performance improvements in healthy elderly participants. This finding suggests that combining non-invasive brain stimulation with MVF could also be a potential interesting tool to counteract the age-related decline in motor function.

Disclosures: M. Hoff: None. **E. Kaminski:** None. **V. Rjosk:** None. **B. Sehm:** None. **C. Steele:** None. **A. Villringer:** None. **P. Ragert:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

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Program#/Poster#: 456.06/RR47

Topic: D.05. Visual Sensory-motor Processing

Support: NSERC

CIHR

Title: Parietal direct current stimulation during sensory-motor learning with reversed vision prevents performance gains and increases in cortical excitability in the untrained hand

Authors: ***M. VESIA**, E. M. STIKSRUD, R. PELLICCIARI, R. CHEN
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Abstract: We previously showed that improved skill performance in the untrained hand after sensory-motor learning with reversed vision was associated with increased excitability in the human primary motor cortex (M1) and parietofrontal circuits in the untrained hemisphere. Neuroimaging has implicated the posterior parietal cortex (PPC) as a critical node in this reversed visuomotor mapping. Here we tested whether transcranial direct current stimulation (tDCS) to the right PPC alters sensory-motor learning with reversed vision. Moreover, we examined whether the degree of sensorimotor learning not only influences the transfer of skill performance in the untrained hand, but also modulates M1 excitability and PPC-M1 interactions in the untrained hemisphere. We measured transfer of skill performance in the untrained left-hand on a serial reaction-time task (SRTT) and cortical excitability with transcranial magnetic stimulation (TMS) in the untrained right hemisphere before and after four training interventions. TMS measures included (i) resting motor thresholds, (ii) motor evoked potential (MEP) amplitude and input-output curves, (iii) intracortical excitability in M1, and (iv) PPC-M1 interactions. The four training interventions included: (1) anodal tDCS to right PPC combined with directly viewing the active learning right-hand; (2) anodal tDCS to right PPC combined with viewing the 'mirrored' image of the moving right-hand superimposed over the inactive hand with left-right optical reversing spectacles (prism); (3) cathodal tDCS to right PPC combined with prism; and (4) sham tDCS combined with prism. Post-training results indicate that both anodal and cathodal tDCS differentially interfered with sensory-motor learning with reversed vision, with larger reduction in accuracy during the cathodal compared to anodal tDCS. We also found that the sham tDCS + prism and anodal tDCS with direct vision during sensory-motor learning with the right hand: (i) increased performance gains in the untrained left-hand; (ii) increased PPC-M1 excitability; and (iii) enhanced M1 'plasticity'. In contrast, both anodal and cathodal tDCS to PPC with reversed vision during sensory-motor learning with the right hand: (i) prevented transfer of skill learning to the untrained left-hand; (iii) decreased PPC-M1

excitability; and (iv) interfered with M1 'plasticity'. These results suggest that the right PPC operates at an optimal excitability level and modulations of excitability interfere with the neural circuits that gives rise to the performance gains and increases in M1 excitability in the opposite hand after sensory-motor learning with reversed vision.

Disclosures: **M. Vesia:** None. **E.M. Stiksrud:** None. **R. Pellicciari:** None. **R. Chen:** None.

Poster

456. Direct Current Stimulation

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Program#/Poster#: 456.07/RR48

Topic: D.17. Voluntary Movements

Support: ERC Grant CTHC

Title: Transcranial direct current stimulation over left angular gyrus decreases the perceptual association between actions and outcomes

Authors: ***N. KHALIGHINEJAD**, P. HAGGARD
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Abstract: The sense of controlling one's own action and through them events in the outside world is referred to as sense of agency (SoA). The sense of agency is fundamental to normal human mental function, but its neural basis is poorly understood. We designed a series of studies using transcranial direct current stimulation (tDCS) to modulate the key brain circuits underlying the control of action. We assessed how these interventions changed the perceived relationship between a voluntary action and its sensory outcome that is believed to be an implicit measure of agency. 55 healthy volunteers, 18-35 years of age (25 females) were tested in three separate experiments. Subjects were asked to look at a clock hand rotating on a computer screen and to make judgements, in separate blocks, about the time of their action, or of a beep which followed their action after 250ms. These were compared to judgements of actions alone, or beeps alone to calculate the perceptual shift of action toward outcome and vice versa. tDCS was applied to specific cortical areas during the task. Experiment 1 compared anodal stimulation of the left angular gyrus (AG) and left dorsolateral prefrontal cortex (dLPFC). In Experiment 2, anodal stimulation of the left AG was compared with cathodal stimulation of the same area. Experiment 3 used a biparietal montage, with anode and cathode over AG of either hemisphere. Participants perceived the beeps as shifted towards the action that caused them, relative to control conditions

with beeps but no actions. In the first experiment, this 'intentional binding' was diminished by anodal stimulation of the left AG but not left dLPFC. Experiment 2, using a different cathodal placement, found a similar but now non-significant pattern. Results of the Experiment 3 replicated the results of the first experiment and also suggested that left, but not right AG is mainly responsible for action-outcome linkage. This is the first causal study of implicit sense of agency of which we are aware. Our results show that association of action and outcome involves the left angular gyrus, possibly following comparison of intended and actual consequences of action. Our anodal stimulations may have artificially activated this mismatch detection process, resulting in an attenuated perceptual shift of outcome toward action that we use as an implicit marker of sense of agency.

Disclosures: N. Khalighinejad: None. **P. Haggard:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 456.08/RR49

Topic: D.05. Visual Sensory-motor Processing

Title: Enhancing the mirror illusion with transcranial direct current stimulation

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Abstract: The perceptual-motor system's estimate of the current arm position is strongly dependent on visual feedback. One compelling example of this phenomena is the mirror illusion (MI). To experience the MI, a participant places her arms on either side of a vertically-oriented mirror, with only the arm on the reflective side of the mirror being visible. When the mirror is placed midway between the two arms, the reflection of the visible arm (viewed in the mirror) appears to be in the same location as the arm behind the mirror. Thus, the MI setup creates a compelling illusion in which visual feedback about movement of the arm behind the mirror comes from movement of the visible arm. This false visual feedback from the MI has been shown, over multiple sessions, to reduce post-stroke movement deficits. During this "mirror therapy", the impaired limb benefits from the illusory visual feedback from the unimpaired limb. Thus, it is highly probable that over time the MI leads to changes in bilateral interactions between motor networks within the two hemispheres. We hypothesized that the MI would be affected when we modified interhemispheric interactions using transcranial direct current

stimulation (tDCS). Specifically, we tested whether bilateral tDCS stimulation to the primary motor cortices (anode-right-cathode-left and anode-left-cathode-right) would modify the MI, as measured using a previously-developed method of objectively quantifying the strength of the MI (Holmes & Spence, 2005). In this method, participants make reaching movements with the unseen arm behind the mirror while viewing the reflection of the other arm. When an offset in the positions of the two limbs relative to the mirror is introduced, the effects of the mirror are manifested in the bias of the reflected arm's position reaching of the unseen arm. We found that active tDCS in the anode-right-cathode-left montage increased the magnitude of the MI relative to sham tDCS. We take these data as a promising indication that tDCS could improve the effect of mirror therapy in patients with hemiparesis.

Disclosures: S. Jax: None. **D. Rosa-Leyra:** None. **H. Coslett:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 456.09/RR50

Topic: D.18. Brain-Machine Interface

Support: AFRL Contract FA8650-11-C-6157

Title: Alternatives to water for the conductance of current during transcranial direct current stimulation

Authors: J. H. KANE[1], M. S. SHERWOOD[1,2], J. G. PARKER[1,2], *M. P. WEISEND[1]
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Abstract: Transcranial direct current stimulation (tDCS) is a method that shows promise in treating neurological and psychiatric disease as well as enhancing performance in healthy individuals. tDCS is applied most frequently with wetted sponge electrodes. However, preparations that utilize water are unstable over long periods of application. Hydrogel (provided by Advanced Brain Monitoring, Carlsbad, CA, USA) and SignaGel (Parker Laboratories, Fairfield, NJ, USA) were evaluated as alternatives to water for the delivery of tDCS. SignaGel and Hydrogel are used routinely in electroencephalography applications. SignaGel has been used as the conductor at the skin electrode interface in multiple works that show behavioral enhancement in healthy controls (McKinley et al, 2013, 2014). Thirty-minute sessions of tDCS at 2 mA were simulated using an ActivaDose (ActivaTek, Inc., Salt Lake City, UT, USA) constant current delivery unit. Current was delivered across a wetted sponge with SignaGel

and/or Hydrogel to conduct electricity at the electrode sponge interface. The voltage was recorded at 15-second intervals during the 30-minute periods of stimulation within the anode, within the cathode, and across continuous bridges of SignaGel or Hydrogel that connected the anode and cathode. The voltage measurements were used to calculate the impedance of the Hydrogel or SignaGel as a function of time during simulated tDCS. SignaGel showed steady, low, replicable impedance over the 30-minute application of current. In contrast, the impedance of Hydrogel was higher and more variable within and between stimulation sessions. During long periods of current stimulation, an unfavorable interaction occurred within Hydrogel resulting in extremely high impedance and the formation of gas bubbles around the electrodes. This interaction was not further explored in this work, but led us to conclude that the electrical properties of Hydrogel are not ideal for tDCS experiments. SignaGel, however, showed reliably low impedances making it a favorable alternative to water preparations.

Disclosures: J.H. Kane: None. **M.S. Sherwood:** None. **M.P. Weisend:** None. **J.G. Parker:** None.

Poster

456. Direct Current Stimulation

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Topic: D.18. Brain-Machine Interface

Support: NHMRC Project Grant 1055084

Australian Postgraduate Award (APA)

UNSW Research Excellence Award

Title: Non-invasive cervical direct current stimulation produces changes in the human motor pathway

Authors: *S. FITZPATRICK[1,2], J. L. TAYLOR[1,2]
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Abstract: Introduction: Direct current stimulation (DCS) of the motor cortex can change neuronal excitability and induce long term plasticity[1]. However, little is known about spinal cord DCS (trans-spinal DCS; tsDCS), particularly at a cervical level. A previous study reports that 20 min of cervical tsDCS can increase muscle responses to motor cortical stimuli for up to 2 hours[2].

Here we aimed to further characterize cervical tsDCS. **Methods:** 3mA of DC was passed between 30cm^2 saline soaked sponge electrodes placed on the anterior neck under the chin and posteriorly over the 7th cervical vertebra. TsDCS was delivered with the anode anterior (antA) or with cathode anterior (antC). EMG responses to cervicomedullary stimulation (cervicomedullary motor evoked potentials, CMEPs) and motor cortical stimulation (motor evoked potentials, MEPs) were recorded from biceps brachii and flexor carpi radialis (FCR). MEPs were also recorded from the first dorsal interosseous muscle (FDI). Study 1: Subjects (n = 7; 2F) received 20 min of antA or sham (1 min of tsDCS) stimulation on 2 days. CMEPs and MEPs were elicited before and for 30 min after stimulation. Study 2: Subjects (n = 8; 4F) received 10 min of antA and antC tsDCS on one day (order balanced across subjects). CMEPs and MEPs were recorded before, during and after each period of tsDCS. Area of CMEPs and MEPs were measured for analysis. **Results:** Study 1: Neither CMEPs nor MEPs differed after antA tsDCS compared to sham stimulation. Study 2: During stimulation, there was a significant main effect of polarity (antA vs. antC; $F_{(1,7)} = 33.88$, $p = 0.0006$) and an interaction of time and polarity ($F_{(1.31,9.16)} = 17.94$, $p = 0.001$) on the area of biceps CMEPs. CMEPs increased to 147 ± 41% (mean ± SD) of pre-stimulation controls during antA ($p < 0.05$) and decreased to 42 ± 20% during antC ($p < 0.005$) stimulation. Similarly, FCR CMEPs displayed a significant main effect of polarity ($F_{(1,7)} = 19.16$, $p = 0.003$) and an interaction ($F_{(1.79,12.53)} = 11.51$, $p = 0.002$) with an increase to 142 ± 44% of pre-stimulation controls during antA ($p < 0.05$) and a decrease to 51 ± 24% during antC stimulation ($p < 0.05$). Biceps and FCR CMEPs returned to baseline levels immediately after tsDCS ceased. Surprisingly, MEPs in all muscles were unchanged. **Conclusion:** Our data indicate that cervical tsDCS results in acute changes to the corticospinal-motoneuronal pathway that, in contrast with previous work[2], do not outlast stimulation. While the specific action of tsDCS is unknown, changes in CMEPs but not MEPs are consistent with altered excitability of corticospinal axons. 1. Nitsche & Paulus *Neurology*. 57:1899-1901, 2001 2. Lim & Shin *NeuroReport*. 22:819-823, 2011

Disclosures: S. Fitzpatrick: None. **J.L. Taylor:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: the Grants-in-Aid for Scientific Research (KAKENHI 24680061)

the Grants-in-Aid for Scientific Research (S) (KAKENHI 21220005)

Development of biomarker candidates for social behavior'', carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Transcranial direct current stimulation (tDCS) over the primary motor cortex during training enhances over-night consolidation of newly-learned ballistic thumb skill

Authors: *S. KOYAMA[1], S. TANAKA[2], S. TANABE[3], N. SADATO[1]
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Abstract: Purpose/Hypothesis: Transcranial direct current stimulation (tDCS) of the brain is a noninvasive technique that modulates cortical excitability in humans. Recent studies have indicated that tDCS over M1 during motor training can enhance consolidation of sequential finger tapping skill (Kantak et al., Eur J Neurosci 36, 2710-2715, 2012). However, it remains unknown whether tDCS could enhance consolidation of ballistic thumb movements, an essential motor skill in daily life and rehabilitation setting. Previous studies indicate that the primary motor cortex (M1) is relevant to ballistic thumb movements learning (Muellbacher et al., Nature 415, 640-644, 2002). Thus, in the present study, we tested a hypothesis that tDCS over M1 could enhance the consolidation of ballistic thumb movements skill. Materials/Methods: Twenty-two healthy subjects (8 females, 25.9±2.2 years) participated in the experiment. The study employed a single-blind, sham-controlled and between-group experimental design. All subjects first trained a ballistic thumb movement with their left thumb (Muellbacher et al., 2002) during tDCS or sham stimulation. In the tDCS group, subjects simultaneously received 1mA anodal tDCS over the contralateral M1 and cathodal tDCS over ipsilateral M1 for 25 min during training (Vines et al., BMC Neurosci 9, 103, 2008). The subjects also performed the same ballistic thumb movements' task 1 and 24 hours after tDCS. In the sham group, the other eleven subjects underwent the identical training sessions except that tDCS were applied only for the first 15s. The primary measurement was the improvement in the peak acceleration of thumb movements 1 and 24 hours after tDCS. Wilcoxon rank sum test were used to compare the improvements in the peak acceleration 1 and 24 hours after stimulation between the tDCS and sham groups. Results: The improvements in the peak acceleration 1 hour after the stimulation were not significantly different between the tDCS and sham group ($p=0.13$). However, the improvements 24 hours after the stimulation were significantly higher in the tDCS group compare to sham group ($p<0.05$). Discussion/Conclusions: tDCS over the bilateral human M1 enhances the overnight consolidation of ballistic thumb movements skill. The present result raises the possibility that tDCS over M1 combined with rehabilitation training might improve ballistic thumb movements in subcortical stroke patients.

Disclosures: **S. Koyama:** None. **S. Tanaka:** None. **S. Tanabe:** None. **N. Sadato:** None.

Poster

456. Direct Current Stimulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 456.12/SS3

Topic: F.01. Human Cognition and Behavior

Title: The effect of low current brain stimulation on performing statistical calculations

Authors: *R. A. HOUSER[1], S. THOMA[1], M. STANTON[2]
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Abstract: The aim of this study was to determine whether the use transcranial direct current brain stimulation (tDCS) impacted statistical calculation. There were three conditions, two experimental and one control. The control group was connected to the tDCS stimulator for the sham condition. The two experimental groups received either a 1 mA or 2 mA stimulation for 20 minutes with the anodal electrode targeting the intraparietal sulcus (P3 in the 10-20 EEG system).. The negative electrode, cathode stimulation, was placed at right middle temporal gyrus (T4 in the 10-20 EEG system) . We used a posttest only research design with random assignment to groups, three groups. There were 34 participants in the study, 10 in each of the experimental groups and 14 in the control group. After the 20 minute stimulation each group viewed an instructional video on calculating a Kruskal-Wallis test (a test to determine differences in rankings). Participants had an opportunity to view the video as long as they chose and as many times as they chose. Paper and pencils were available for note taking. After viewing the video each participant was given a single statistical problem, calculating a Kruskal-Wallis test on a given set of data. . The dependent variable was performance on a statistical calculation, the Kruskal-Wallis test. Scoring of the statistical test was initially done without any identification of the group assignment, e.g. control, 1 mA or 2 mA. Participants were awarded points for completion of five steps in the calculation (e.g. identifying the critical value and calculation of the sum of ranks) for a range of scores between 0 and 5 points. A one-way ANOVA was calculated comparing the three groups' calculation scores. The mean of the control was .78 with a standard deviation of 1.12. Those in the 1 mA group had a mean of 1.60 with a standard deviation of 1.43. Finally the 2 mA group had a mean of 2.30 with a standard deviation of 1.77. Significant differences were found between the three groups, $F(2, 31)= 3.36$ with a $p=.048$. Post hoc Scheffe results found that there were significant differences between the control group and the 2 mA.Group, the 2 mA group had significantly higher scores on the statistical calculation compared to the control group. No other significant differences were found between the other groups. Based on these results it appears that a higher low current brain stimulation over the left

intraparietal sulcus positively impacts performance on statistical calculations. This research was a pilot and the sample size was relatively small, 34 participants, and more research needs to be conducted.

Disclosures: R.A. Houser: None. **S. Thoma:** None. **M. Stanton:** None.

Poster

456. Direct Current Stimulation

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Program#/Poster#: 456.13/SS4

Topic: D.17. Voluntary Movements

Support: NIH Grant T32 HD007414-16

NIH Grant R01 HD053793

Title: Effect of SI stimulation on visuo-proprioceptive realignment

Authors: *H. J. BLOCK[1], P. CELNIK[2,3,4]
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Abstract: The brain is thought to weight and combine proprioceptive and visual information to control voluntary movement. Any disruption to proprioception, as frequently happens in stroke and other conditions, is therefore likely to affect multisensory processes. This could impair a patient's ability to adapt his movements in a changing environment. For example, when reaching into a sink full of water, the brain's visual estimate of hand position is shifted from its proprioceptive estimate by the water's refraction of light. While movement errors may occur initially, a healthy person quickly learns to compensate. For example, the brain can shift the proprioceptive estimate closer to the visual estimate (proprioceptive realignment). Although failure to compensate for a perturbation can result in movement difficulties, little is known about how changes in proprioception affect multisensory processing. Here we used transcranial direct current stimulation (tDCS) to investigate the role of primary somatosensory cortex (SI), where proprioceptive processing occurs, in visuo-proprioceptive realignment. Subjects participated in two experiments (n=15) each containing two sessions of reaching movements with their right hand to visual (white square), proprioceptive (left fingertip), or combined targets, without direct vision of either hand. In one session, excitatory (anodal) tDCS was applied over right SI. In the

other session, sham tDCS was applied. We imposed a 7cm misalignment between vision and proprioception related to the left hand by moving the white box away from the left fingertip. In Experiment 1 subjects realigned proprioceptive estimates of left hand position *more* during anodal tDCS than during sham. Experiment 2 represented a different environmental context, in which proprioceptive target salience was decreased by placing the target finger on a large plush ball rather than directly beneath the reaching surface. In this situation, anodal tDCS caused subjects to realign proprioception *less* than during sham. I.e., the change in proprioceptive target salience reversed the effect of SI tDCS on proprioceptive realignment. This context-dependent effect may reflect homeostasis in the cortex, where the prior cortical state influences the direction of change to keep cortical activity within a certain range. Answering this question will advance our understanding of the functional and neural basis of visuo-proprioceptive realignment and will help lay the groundwork for future treatments of patients with proprioceptive deficits resulting from stroke, neuropathy, and other conditions.

Disclosures: H.J. Block: None. **P. Celnik:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 457.01/SS5

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP 97821

Title: Testing orbitofrontal contributions to formation of a value-based attentional set in a two dimension probabilistic reversal-learning task

Authors: *A. R. VAIDYA, L. K. FELLOWS
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Abstract: Everyday life can present difficult choices in noisy environments: whether deciding between cereals in a crowded supermarket, or stocks on a trading floor. Making appropriate decisions in such situations requires attending to motivationally relevant features, while ignoring the irrelevant aspects of available options. Many studies have shown that humans and animals with focal damage to orbitofrontal cortex are impaired in making optimal choices based on fluctuating stimulus-reward associations. However, the mechanism by which OFC contributes to such decisions is not well understood. In the current study, we tested whether damage to this

region in humans affects the ability of patients to learn to selectively associate reward with one stimulus dimension in the presence of a second, distracting dimension. We tested patients with OFC damage, a matched group of patients with prefrontal damage excluding OFC, and healthy controls in a probabilistic reversal-learning task where options were compound stimuli with two dimensions (shape and color). Critically, only one dimension was predictive of reward. Healthy participants were able to learn the value of the relevant dimension, showing only a slight tendency to be distracted by the second, irrelevant dimension that waned as they accrued experience. In contrast, OFC damaged patients learned about the predictive and non-predictive dimensions almost equally, though they were no more distracted by the irrelevant dimension than controls. These patients were also slower to tune learning to the relevant dimension as they gained experience. These results suggest a role for OFC in forming an attentional set that guides learning about value. In this way, OFC may contribute to decision-making by biasing attention to valuable features in the environment.

Disclosures: **A.R. Vaidya:** None. **L.K. Fellows:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: Killam post doc to CSC

NSERC DG to JTE

Title: Action success, not reward value, governs trial-by-trial biases during rapid reach planning

Authors: *C. S. CHAPMAN[1], J. P. GALLIVAN[2], J. T. ENNS[3]
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Abstract: We tend to allocate more cognitive resources (e.g., attention) to objects conferring some benefit. Interestingly, a positive reward can be associated with an object after only one exposure. Behavioural evidence for this single-trial learning comes from recent work by Hickey & van Zoest (2012) showing that a successfully fixated target, which randomly received a large

positive reward on a previous trial, will bias fast saccadic eye movements toward its location on the next trial, even when it is a to-be-avoided distractor. Here, we tested how these transient reward associations would bias rapid reaches toward targets that could result not only in rewards but also in penalties. Fifty-five participants performed rapid reaches (as in Chapman et al. 2010) toward target displays containing two shapes, an "X" and an "O". Participants randomly received +1 point (neutral) on 2/3 of trials, +10 points (positive) on 1/6 of trials and -10 points (negative) on 1/6 of trials, when they completed a rapid reach toward the cued shape within pre-specified time constraints. Our analysis focused on trials immediately following each reward type and compared cases in which the shapes switched spatial locations versus remained in the same location. We found rapid reach behaviour consistent with the previous eye-movement work: the hand was drawn toward the target shape if it had previously been rewarded. However, in our study, we found that the hand was drawn to the target shape regardless of whether the reward was positive, neutral or negative. Notably, there was a fourth outcome that participants could experience: if subjects hit the cued target but failed to meet the timing criteria, they received a "Too Slow" error (this occurred on a significant number of trials). Critically, following these trials, we found no evidence for any bias toward the previously cued shape. These results suggest that what participants experience as rewarding or punitive can be highly context-specific. Based on the nature of the reaching biases observed, we show that the reward associated with the successful completion of the trial (i.e., executing the correct action) can supersede the processing of reward value.

Disclosures: C.S. Chapman: None. **J.P. Gallivan:** None. **J.T. Enns:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 457.03/SS7

Topic: F.01. Human Cognition and Behavior

Title: Human conditioned place preferences using secondary reinforcers

Authors: *R. S. ASTUR, A. PALMISANO, A. CAREW, B. DEATON, F. KUHNEY, R. NIEZRECKI, E. HUDD, K. MENDICINO, C. RITTER
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Abstract: Recently, humans have been conditioned to prefer a virtual reality place that has been paired with primary reinforcers, such as food, drugs, and music. This is advantageous in that it

allows for a more thorough understanding of the factors involved in addictions. Our current goal was to examine whether similar conditioning could occur using a secondary reinforcer that provided little obvious reward to the participants. Ninety-five undergraduates were recruited for a 60 min study. Participants were placed into a VR environment consisting of 2 visually distinct rooms connected by a door. Throughout the experiment, there was a counter/scoreboard at the top of the VR world. For the conditioning sessions, participants underwent 6 pairing sessions in which they were locked into one of the two rooms and were to explore the VR environment by using a joystick to move throughout the VR world. While in room **A**, 50-100 points occasionally were added to the VR counter, independent of participants' actions. In total, about 17 of these point rewards were administered. In Room **B**, the counter stayed at 0 for the entire session. Room / Points pairings and ordering were counterbalanced. After a short break, a test session was administered, and participants were given free access to the entire VR environment for 5 min, and no point rewards were administered. On the test day, we observe that participants display a significant place preference by spending 55% of the time in the room previously paired with point rewards compared to 45% in the room paired with no rewards ($p < 0.05$). Hence, participants display a significant conditioned place preference for a VR room previously paired with a secondary reinforcer that provided them with no obvious reward. Additionally, participants explicitly rate the points room higher on an enjoyment scale than the no-points room ($p < 0.05$). When we used a gaming experience scale to stratify participants, we see that those who are in the upper quartile of gaming experience show no conditioned place preference, whereas those in the bottom quartile show a highly significant place preference (66% point room vs. 33% no-points room, $p < 0.001$). Hence, we see that conditioned place preference in humans extend to environments paired with secondary reinforcers. Moreover, gaming experience may be a critical factor in learning paradigms presented within virtual reality. Future research will examine extinction, reinstatement, and other learning phenomena within virtual reality.

Disclosures: **R.S. Astur:** None. **A. Palmisano:** None. **A. Carew:** None. **B. Deaton:** None. **F. Kuhney:** None. **R. Niezrecki:** None. **E. Hudd:** None. **K. Mendicino:** None. **C. Ritter:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Program#/Poster#: 457.04/SS8

Topic: F.01. Human Cognition and Behavior

Support: ECGPS grant (Internal funding)

NIH RO3 - DA031583

Title: Dopamine is necessary for reward-related incidental learning improvements: Evidence from patients with Parkinson's disease

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Abstract: Midbrain dopamine neurons have been demonstrated to respond to the both the presence of an unexpected reward and the absence of an expected reward. This dopamine reward-predication signal has been shown to be involved in various forms of learning including incentive learning and instrumental learning. Recently however, Freedberg and colleagues have demonstrated that rewards can be used to bolster incidental learning of associations, even when participants demonstrate little to no awareness of which associations were rewarded. Here we test the hypothesis that dopamine is necessary to see these reward-related improvements by comparing learning and performance differences between patients with Parkinson's disease (PD), who experience an accelerated decrease in dopamine neurons with age, to age-matched controls. Participants in this study performed a single-session experiment where they were asked to respond to combinations of faces in which half of the combinations were deterministically linked to a monetary reward. Immediately following training of the rewarded and unrewarded combinations participants entered a transfer block where they were asked to perform the same combinations without any feedback or rewards. During the transfer block Galvanic skin responses (GSRs) were measured in response to both previously rewarded and unrewarded combinations. Our preliminary data provide evidence that patients with PD experience significantly less reward-related incidental learning improvements compared to age-matched controls. These results may emphasize the critical role of dopaminergic transmission in acquiring rewarded information as well as highlight the potential learning deficits of patients with PD in acquiring rewarded information.

Disclosures: M.V. Freedberg: None. **E. Hazeltine:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Program#/Poster#: 457.05/SS9

Topic: F.01. Human Cognition and Behavior

Title: Electrophysiological correlates of violations of consumers' price expectations in a simulated shopping task

Authors: *A. SCHAEFER[1,2], L. G. BURATTO[2]
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Abstract: Differences between actual and expected prices are widely known to be a major determinant of purchasing decisions. However, the neurobiological underpinnings of this phenomenon are still largely unknown. We present here a study that tested whether neural systems of error monitoring were involved in the detection of deviations between expected and actual prices in a simulated shopping task. Forty participants viewed a series of pictures of desirable products that were priced at levels above or below participants' expectations in four main conditions: Underpriced-Large deviation (UL), Underpriced-small deviation (US), Overpriced-Large deviation (OL), Overpriced -small deviations (OS). In order to measure neural activity related to error monitoring, we used the Feedback-Related Negativity (FRN), a brain event-related potential (ERP) measured with scalp EEG. The FRN is widely known to be a neural index of prediction error and it is driven by neural generators located in medial frontal areas (Alexander & Brown, 2011; Walsh et al., 2012). Behavioural results indicated significant differences across conditions in "Buy" rates: UL>US=OS>OL. Furthermore, we found that FRN amplitude successfully indexed price deviations: FRN amplitudes were less negative in UL compared to OL trials. In addition, this effect was primarily driven by an effect of positive prediction error: FRN amplitudes were less negative for UL compared to US trials. These findings show that the FRN can successfully track when prices are "better than expected" in a simulated shopping task, and they also suggest that neural systems of error monitoring play an important role in the formation of consumers' expectations. References: Alexander WH, Brown JW (2011) Medial prefrontal cortex as an action-outcome predictor. Nat Neurosci 14: 1338-1344. Walsh MM, Anderson JR (2012) Learning from experience: Event-related potential correlates of reward processing, neural adaptation, and behavioral choice. Neuroscience & Biobehavioral Reviews 36: 1870-1884.

Disclosures: A. Schaefer: None. **L.G. Buratto:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Program#/Poster#: 457.06/SS10

Topic: F.01. Human Cognition and Behavior

Support: R01MH098861

5T32MH065214

Title: Is model fitting necessary for model-based fMRI?

Authors: R. C. WILSON, *Y. NIV
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Abstract: Model-based analysis of fMRI data is an important tool for investigating the computational role of different brain regions. With this method, theoretical models of behavior can be leveraged to find the brain structures underlying variables from specific algorithms, such as prediction errors in reinforcement learning (RL). Typically, the analysis involves generating regressors of the quantity of interest from a model of the task dynamics, and regressing these against brain activations acquired using fMRI. One potential weakness with this approach is that model-based regressors, and hence the results of the analysis, often depend on free parameters of the model. These are usually determined by fitting the model to behavioral data, but if the fit parameters are wrong we might draw very different conclusions than if the "correct" parameters were used. In this work, we tested to what extent incorrectly fitting parameters changes the results of model-based fMRI. We focused on RL regressors for value and prediction error and examined their sensitivity to the learning rate parameter both in theory and in previously published datasets. Surprisingly, we found that gross errors in estimating the learning rate lead to only minute changes in the results. This was because the value and prediction error regressors themselves were relatively insensitive to the specific setting of the learning rate. For instance, consider the prediction errors generated in a simple experiment in which subjects earn $1 or $0 with 50% probability on each trial. Regardless of our choice for the learning rate, a $1 reward will always lead to a positive prediction error and a $0 reward will always result in a negative prediction error. Thus, even with a large error in the fit learning rate, the prediction error regressor will strongly correlate with the "true" prediction error signal in the brain, and the regression will still pick up activity in the brain areas representing this true prediction error. Our findings thus suggest that precise model fitting is not always necessary for model-based fMRI. They also highlight the difficulty in using fMRI data for arbitrating between different models or model parameters, and suggest methods to optimize experimental design to obtain higher or lower sensitivity to parameters, as required. Finally, we stress that our results apply only to prediction error and value regressors in simple RL models. Other models will need to be evaluated on a case-by-case basis and our theoretical analysis shows how this can be done.

Disclosures: R.C. Wilson: None. **Y. Niv:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: Chinese Academy of Science Grant XDB02050500

Title: The neural correlates of probabilistic and volatile rewards during decision making

Authors: H. WANG, M. SHU, Y. CHEN, T. CHEN, C. XU, Z. WANG, *T. YANG
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Abstract: Decision making often relies on probabilistic and volatile reward feedbacks, yet it is not well understood how the brain processes such information to achieve learning. Previously we reported that orbitofrontal (OFC) neurons in macaque monkeys encoded multiple aspects of reward information, including magnitude, probability and volatility. To explore their neural correlates in a broader network of brain areas, we trained a group of healthy human subjects with a similar visual cue-reward association task. In the task, simple geometric shapes were shown on a computer screen, and the subjects had to guess whether the shapes were associated with 1 point or 5 points by pressing buttons. If they guessed correctly, they would gain corresponding points. Incorrect guesses would be penalized and corresponding points would be deducted. A set of 6 visual cues were used, including 2 fixed cues that were always associated with 1 and 5 points, 2 probabilistic cues that were probabilistically associated with either 1 or 5 points at fixed probabilities, 1 volatile cue that predicted points that randomly flipped between 1 and 5 every 40 trials on average, and 1 volatile probabilistic cue indicating points at probabilities varied every 40 trials on average. All cues were randomly interleaved during a session. The probabilities and changing rates were not explicitly told to the subjects. They had to learn through trial-and-error and were instructed to try to get as many points as possible. The set of cues and the reward associations were fixed for each subject throughout the experiment. The subjects took three to four training sessions, each of which included 250 trials. After the training, the subjects gave consistent responses to the non-volatile cues, and adjusted their responses to the volatile cues based on feedbacks. We then scanned the subjects in a 3T MRI scanner while they were performing the task. We identified the neural correlates of the reward aspects involved in the task. First, the activity of ventromedial prefrontal cortex, globus pallidus (GP), insular cortex (IC), and posterior cingulate cortex were correlated with the magnitude of reward value (fMRI effects reported at $p<0.001$). Second, anterior cingulate cortex (ACC), GP, ventral striatum (VS) and IC signaled the cues' volatility. When the reward cue was probabilistic, the activity of VS

and GP reflected the reward prediction error only when the cue was also volatile. Lastly, striatum, ACC and medial OFC were activated by probabilistic cues. Our results suggest that distinct neural circuits may underlie different aspects of reward processing in a contextual dependent manner and contribute to decision making.

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Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Young Scientists (B), MEXT, Japan

National Institute of Information and Communications Technology

Title: Human associative learning is enhanced by the subliminal emotional primer

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Abstract: Our associative learning in daily life is not only affected by the rational factors such as reward amounts or probabilities, but also often affected by the conscious and subconscious emotional factors. Recently we showed that the emotional factor, which was easy to be conscious, enhanced the learning rate and the enhancement was explained by ventral striatum-amygdala Interaction (Watanabe et al., 2013). However those emotional effects were not always conscious phenomena. We sometimes cannot be conscious the emotional states by ourselves. In this study, we focused on the subconscious mechanism of the enhancement of the learning by the emotional factor with behavioral experiments and computational approach. In the experiments, we presented task-independent fearful or neutral facial primers prior to the cue subliminally or supraliminally to modulate the emotion of the participants (n = 91), when they engaged in the probabilistic reward learning paradigm. As results, the subliminal (0.027 or 0.033 seconds duration with masks) primers enhanced the learning rates (Sublim Fear > Sublim Neutral; n = 40

t39 = 3.359, p = 0.007) and negatively biased 100 yen choice more effectively compared with the supraliminal (0.040 and 0.047 seconds duration) primers (Sublim Fear (n = 40) < Supra Fear (n = 51); t85.605 = -2.676, p = 0.036). Although the enhancements of the learning rates were robust even if we tested based on the presented durations; 0.027 sec. (n = 20, t19 = 2.211, p = 0.040), 0.033 sec. (n = 20, t19 = 2.482, p = 0.023), and 0.047 sec. (n = 20, t19 = 2.194, p = 0.041), there was no statistical difference only in 0.040 sec. duration (n = 31, t30 = -0.560, p = 0.580).Thus, there was a valley for the emotional enhancements. We discussed the neural mechanism behind the valley based on the subcortical and cortical visual pathway theory.

Disclosures: **N. Watanabe:** None. **M. Haruno:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Program#/Poster#: 457.09/SS13

Topic: F.01. Human Cognition and Behavior

Support: NSERC 312409-05

Title: Reward feedback stimuli elicit high-beta oscillations in human dorsolateral prefrontal cortex

Authors: ***A. HAJIHOSSEINI**, C. B. HOLROYD
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Abstract: Previous research has shown that presentation of reward feedback stimuli elicits a burst of power in high-beta EEG oscillations (20-30 Hz) over the frontal areas of the scalp in humans (Cohen, et al., 2007; HajiHosseini, et al., 2012; Marco-Pallares, et al., 2008). This activity is reported to be sensitive to valence and probability of the feedback, properties that might indicate an important role in learning from feedback; however the source and function of this oscillatory activity are poorly understood. To investigate these issues we recorded EEG from the scalp of twenty-six volunteer participants while they navigated through a virtual T-maze to obtain monetary reward. A stimulus cue was presented on the screen at the beginning of each trial that indicated one of four possible response-reward mapping probabilities: high or low probability of reward in either the right or left alley, where the alternative alley was never rewarded. Following cue presentation on each trial participants made either a right or left response, followed by a view of the end of the selected alley that showed either a reward (5 cents) or no-reward (0 cents) abstract feedback stimulus. Participants were not informed about the mapping and were instructed to determine by trial and error the best alley to select for each

cue. Our results revealed that feedback presentation elicited a burst of power in the beta frequency range that, consistent with previous studies, was significantly higher for reward compared to no-reward feedback ($F(1,25) = 18.25$, $p < 0.001$), but no effect of probability or interaction with probability was observed. Further, source localization using sLORETA revealed that the maximal valence contrast in beta power was localized to right dorsolateral prefrontal cortex (DLPFC) ($t= 5.84$, $p=0.001$). Previous theories about DLPFC propose that this area plays a role in representing task sets (Sakai, 2008) and in regulating cognitive processes that depend on working memory (Barbey et al., 2013). Further, recent findings demonstrate that beta oscillations recorded in the local field potential from monkey prefrontal cortex are associated with a mechanism that selects neural ensembles specific to the task at hand (Buschman et al., 2012). In this context, our results suggest that reward-related beta oscillations produced in human DLPFC reflect a role for it in coordinating the activity of neural areas that learn action sequences to maximize reward.

Disclosures: A. Hajihosseini: None. **C.B. Holroyd:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH085092

Title: Perceptual salience and reward both influence feedback-related neural activity arising from choice

Authors: *B. LOU, P. SAJDA
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Abstract: For day-to-day decisions, multiple factors influence our choice between alternatives. For example, reward value and visual salience can each, individually, strongly affect the underlying cognitive processes of decision formation. Previous research using EEG has shown that feedback-related negativity (FRN) components are sensitive to reward valence and probability but are insensitive to reward magnitude. We designed an experiment that systematically changed perceptual salience and reward value, while simultaneously measuring EEG from subjects performing the task. Specifically, we conducted a four-choice task, where

subjects rapidly made choices between images of faces, cars, and houses containing different levels of stimulus noise and associating with different amounts of monetary reward. The reward ratios between the two categories yielding monetary payout (faces and houses) varied from 4 to 1/4 in five steps. The third image type (cars) yielded no payout and served as distractors. Image salience for the two payout categories was negatively correlated with one other and randomized trial-to-trial. As subjects performed the task we recorded 85-channel EEG. We performed a single-trial analysis by defining a series of time windows with different feedback-locked times and used a logistic regression classifier to find the optimal time window for discriminating feedback conditions. Behavioral data showed that selection rates of target images were positively correlated with their corresponding values as well as their perceptual salience, though the slope of the choice curves systematically varied across reward conditions, demonstrating a significant interaction between reward and saliency. Analysis of feedback-locked EEG showed event-related potentials (ERPs) at approximately 300 ms and between 400-500 ms after feedback varied for small vs. large rewards. Source localization (sLORETA) for these two components showed activity in ACC, SMA and IPL, all regions related to reward expectation. Furthermore, single-trial analysis indicated two similar components that maximally discriminated between different feedback conditions and classifier discrimination was significantly increased for larger differences between monetary payout categories. This suggests reward magnitude was not treated in isolation but integrated with saliency information to form choices. Our finding also suggests that subjects try to maximize reward resulting in more reward prediction errors for risky but high reward payouts, and that this strongly influences feedback-related neural activity.

Disclosures: B. Lou: None. **P. Sajda:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Title: Effects of the target setting on the motor skill learning and motivation in healthy people

Authors: *M. HIYAMIZU, H. MAEOKA, A. MATSUO, S. MORIOKA
Kio Univ., Nara, Japan

Abstract: Purpose The purpose of this study was to clarify effects of the target setting as an intrinsic reward on the motor skill learning and motivation compared to monetary reward in

healthy people. Methods Thirty-nine healthy subjects (21 females, 18 males, mean age of 21.6 years) were enrolled in this study. The subjects were assigned randomly to Control, Monetary, and Target groups. Subjects performed the two-ball rotation task by non-dominate hand as many as possible for 20 s. Subjects performed a task in the first, second and third session, and the free-choice period for 3 min was given between each session. All subjects were provided theirs score of the first session and they were given some instructions after first session for each group, respectively. Control group received encouragement to exceed the score in the second session. The monetary group was informed that they would obtain 500 Japanese yen for second session if their score were greater than first. The target group was showed the target board that collected the data from 20 volunteers. In the free-choice time, subjects were left alone in a room and they were allowed to take a break or practice the task. At the end of second session, all subjects were instructed that they performed a task in the third session after the second free-choice period for 3 min. For the third session, subjects in monetary group were informed that they would not obtain any money. In target group, the target board was removed at the end of second session. In the second free-choice time, they were allowed to spend free time. The number of rotation for 20 s was counted and we measured the Time of Self Exercise (TSE) that measured times that subjects voluntary hold two balls by non-dominant hand from video movies in first and second free-choice periods. The number of rotation was analyzed in a 3 group × 3 session by two-way ANOVA and Tukey's test. The difference of TSE (d/TSE) in each group was calculated by subtracting the second free-choice period from the first free-choice period. The d/TSE was analyzed in the Kruskal-Wallis test. Results Control and monetary groups showed a significant difference between the first session and the third session ($p<0.05$). But there were no significant difference between other any sessions. However, the target group showed significant differences between all sessions ($p<0.01$). The d/TSE was no significant differences between 3 groups ($p=0.891$). But the TSE in the target group were greater than other groups in the first and second free-choice periods. Conclusion Our results suggest that the target setting increases amount of self exercise and improve the motor skill learning compared to the monetary reward.

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Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: NIDA R03 DA032580

Title: Dopaminergic midbrain responses to stress inhibit mesolimbic reward processing in humans

Authors: *K. HENNIGAN, S. M. MCCLURE
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Abstract: A growing body of evidence suggests that the midbrain dopamine (DA) system is functionally heterogeneous, with subpopulations differentially encoding positive and negative motivational information. While it is well established that mesolimbic DA activity is essential for reward learning and related behaviors, mesocortical DA activity increases with stress and aversion. Stress has been shown to reduce behavioral and neural sensitivity to rewards, though how stress exerts its influence on reward circuitry remains unknown. One possibility, consistent with known DA anatomy in animals, is that stress-related mesocortical activation is directly related to decreases in mesolimbic reward processing. To examine this relationship, we scanned healthy individuals while they performed an instrumental learning task under both a stress condition (threat-of-shock) and a no-stress condition. The learning task entailed having participants choose between pairs of cues that were associated with probabilistic monetary outcomes. We employed high-resolution functional imaging in conjunction with reinforcement learning modeling to examine activity within the midbrain and DA target regions related to reward learning and the effects of stress. We hypothesized that reward-related mesolimbic activation would be diminished while under stress compared to baseline, consistent with previous studies. We also hypothesized that stress would be associated with increased mesocortical activity (measured as functional connectivity between the DAergic midbrain and prefrontal cortex), and that individual differences in this activity would predict stress-induced changes in mesolimbic reward processing. Taken together, these results provide evidence for functional heterogeneity within the human DAergic midbrain and they suggest that DAergic interactions mediate the effect of stress on reward processing. This work has important implications for understanding how stress alters reward processing and increases vulnerability to addiction.

Disclosures: K. Hennigan: None. **S.M. McClure:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: R01 NS084948

Title: Sensory prediction errors affect reinforcement learning

Authors: *S. D. MCDOUGLE[1], R. B. IVRY[2], J. A. TAYLOR[1]
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Abstract: Thorndike's Law of Effect states that when an action leads to a desirable outcome, that action is likely to be repeated (Thorndike, 1911). However, when an action is not rewarded, the brain must solve a credit assignment problem: Was the lack of reward attributable to a poor decision, an unanticipated event, or weak action execution? For example, if while sipping on a cup of coffee you spill it, the unpleasant sensation should be attributed to a fault in your motor system and not the coffee itself. While this solution to the credit assignment problem is intuitive, there has been little work on this problem. The motor system has specialized neural circuitry to signal sensory prediction errors separate from reward prediction errors, but it is unknown if and how this sensory feedback influences reinforcement learning. To address this potential interaction, we had three groups of 20 healthy subjects perform a two-arm bandit task in which we varied the required action and sensory feedback. In two groups, subjects were required to reach to virtual targets, the "slot machines." We manipulated the payoff of each slot machine through a random walk of points that varied between 1 and 100. Importantly, we also manipulated the difficulty of successfully querying each slot machine by varying the probability that a given reach would hit it. Thus, both the probability of a successful reach and the payoff varied throughout training. In the Cursor condition, subjects saw a cursor that represented the position of their hand, which should maximally engage a system sensitive to sensory prediction errors. In the Binary condition, subjects had information only about the success or failure of their reach. In both conditions, the instructions emphasized that no reward would be earned if a reach missed the target. We found that the Cursor group was biased toward the "risky" target that was harder to hit but yielded higher average rewards. Conversely, the Binary group was risk averse, preferring the "safe" target. In a control condition, subjects responded with button presses instead of reaches, and they were instructed that on some trials the slot machines would fail and yield no reward. The control group displayed robust risk aversion, consistent with previous studies. We modeled our results using a reinforcement learning model, and found that the apparent risk seeking behavior of the Cursor group could be explained by a simple "gating" mechanism, whereby sensory prediction error information about an unsuccessful action discourages the reward system from updating the value of the selected object. These results point to a novel solution for solving the credit assignment problem in reinforcement learning.

Disclosures: S.D. McDougle: None. **R.B. Ivry:** None. **J.A. Taylor:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: NIMH/FIC R21MH095656NIMH/FIC R21MH095656 to MAG

Title: DAT1-COMT gene interaction modulates learning from reward in healthy individuals

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Abstract: Sensitivity to positive feedback varies considerably among individuals. One possible reason for this can be attributed to genetic variability in the dopaminergic system. In particular, a Val158Met substitution in the catechol-o-methyl-transferase (COMT) gene is thought to regulate tonic release, and subsequently phasic release, of dopamine by inducing a 4-fold reduction in its activity, whereas DAT1, a variable number of tandem repeats polymorphism (VNTR) in the dopamine transporter (DAT) gene is thought to regulate phasic release of dopamine by affecting DAT density. To study the cognitive correlates of these physiological processes, we tested 53 healthy subjects using a computer-base cognitive learning task that allows for the dissociation of learning from positive versus negative feedback. We grouped subjects according to their COMT/DAT1 genotype into four groups: (1) Val/Val-9/9, with high COMT activity, low DAT activity, and high tonic and medium phasic dopamine, (2) Val/Val-10/10, high COMT activity, high DAT activity, and high tonic and low phasic dopamine, (3) Met/Met-9/9, with low COMT activity, low DAT activity, and low tonic and high phasic dopamine, and (4) Met/Met-10/10, with low COMT activity, high DAT activity, and low tonic and medium phasic dopamine. Subjects with genotypes reflecting medium phasic dopamine signals learned significantly better from positive feedback than other groups. There was no difference between groups in learning from negative feedback. These findings suggest the importance of looking at collections of genes that work together to mediate the role of dopamine in learning from positive feedback. New insights in the interaction between various dopamine-regulating pathways may be useful in future research to better understand individual differences in brain disorders where dopamine dysfunction is implicated, including Parkinson's disease and schizophrenia.

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Poster

457. Human Learning: Feedback, Reinforcement, and Reward

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 457.15/SS19

Topic: F.01. Human Cognition and Behavior

Support: NSF SBE-0354420 Supplement 0839229

Title: Modulation of declarative memory efficacy affects neither learning rate nor the role of reinforcement learning systems in deterministic, feedback-based decision-making

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Abstract: Feedback about our choices is a crucial part of how we gather information and learn from our environment. Deterministic choice-outcome experiences are generally thought to be encoded and represented by declarative memory processes, but predictions of choice value, generated by reinforcement learning (RL) systems, should be able to guide behavior as well. This study explores the role of RL systems in feedback-based learning of deterministic choice-outcome relationships, while modulating the engagement of the medial temporal lobe (MTL) based declarative memory system. Using whole-brain fMRI, we scanned human subjects while they learned correct responses to a list of 50 or 100 word pairs in a concurrent discrimination learning task. Behavioral choice data were modeled using a model-free RL agent to estimate trial-level reward prediction error (RPE) signals. Together with the manipulation of list length, this allowed us to identify regions of interest associated with reinforcement learning (i.e., RPE effects) and with declarative memory processes (i.e., list length effects). Behaviorally, the rate of learning during the task was unaffected by list length, as was the ability for the RL agent to predict subjects' choices. Imaging results showed a clear link between activity in the basal ganglia (BG) and behavioral RPE signals and that activity in the BG did not vary by list length. Critically though, regions in the medial temporal lobe (MTL), including the parahippocampal

gyrus, modulated with list length; activity was robust for the shorter list (50 pairs) and absent for the longer list (100 pairs). Analysis of subsequent memory effects on these regions showed that activity in both the BG and MTL were predictive of memory confidence but only activity in the MTL modulated by memory strength. These findings suggest that MTL-mediated processes contribute to the strength of formed memories, but that RL-based processes are crucial for the learning of deterministic, declarative choice-outcome information. Altogether, this study highlights that interactions between declarative and RL systems are critical for optimal learning and illustrates the importance of reinforcement learning for declarative memory tasks that involve deterministic feedback relationships.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH F31DC013500-01

Title: Reward-related modulation of conditioned stimulus representations after associative learning

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Abstract: An essential function of the human brain is to encode associations between sensory cues and their related outcomes, which are then used to guide adaptive behaviors and maximize available resources. Studies of reward-related neural processing tend to overlook the importance of the identity of predictive sensory cues themselves, which are critical for tethering learned reward value to the appropriate predictive stimulus. Here we conducted an experiment in which human participants (N = 15) underwent a Pavlovian associative learning paradigm involving four olfactory cues as conditioned stimuli (CS) and monetary reward values as unconditioned stimuli (US). Before and after learning subjects provided ratings of odor pleasantness and perceptual similarity between each possible odor pair, and underwent fMRI scanning while performing an odor detection task on the four odor cues. During the learning phase two of the odors (CS+) were

paired with $1.00 rewards with 100% contingency and the remaining two were never paired with reward (CS-). Analysis of the behavioral ratings revealed that the two CS+ were rated as significantly more pleasant after learning when compared to the CS-. Moreover, there was a significant increase in rated perceptual similarity between the two CS+ after learning. Both of these effects were directly related to the degree of learning during the conditioning phase, indicating that the reward pairing induced both an increase in value and a modulation of perceptual similarity for the CS+. Analysis of the fMRI data using multivoxel pattern techniques revealed that at the level of primary olfactory (piriform) cortex, representations evoked by the odor cues principally reflected perceived similarity between the odors in both the pre-learning and post-learning scanning phases. However, after learning these representations were modulated to reflect the acquired reward value along a secondary dimension. Conversely, in the orbitofrontal cortex (OFC), odor-evoked representations were not related to perceptual similarity, but were updated after learning to reflect the acquired reward value along a principal dimension. These results shed light on how changes in the predictive reward value of conditioned olfactory cues are represented at multiple levels of the sensory processing hierarchy.

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Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01GQ1003B

Title: Neural correlates of contingency learning in contextual fear conditioning

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Abstract: Differential contextual fear conditioning in humans requires subjects to learn the association between a specific context (CS) and an aversive stimulus (US) in order to predict which context constitutes a threat. To date little is known about how subjects who learn the CS-US contingency differ on a neural level from non-learners. In contrast to previous studies, which used context-stimuli that were distinguishable by single elements, we employed a paradigm

which uses two feature-identical contexts that only differ in the arrangement of the features. This paradigm necessitates configural processing of the contexts and due to the demanding nature of the stimuli resulted in comparable groups of contingency aware and contingency unaware subjects. We used functional magnetic resonance imaging to investigate the acquisition of contextual fear in a sample of 94 young healthy adults. The experimental procedure consisted of three conditions: one picture that was never associated with an electric stimulus (CS-) and a second picture where a shock was administered in 50 percent of the trials (CS+paired and CS+unpaired, respectively). A two sample t-test between the groups "contingency aware (n = 43) > contingency unaware (n = 51)" with the first-level contrast "Cs+unpaired > CS-", revealed stronger BOLD responses in bilateral anterior insula, bilateral inferior frontal gyrus, superior medial gyrus and the ventral striatum. The opposite contrast: "contingency unaware > contingency aware" showed stronger activations in middle orbitofrontal cortex, right hippocampus and precuneus. These results show that brain regions associated with the anticipation of an aversive event, like anterior insula, were only active in contingency aware subjects, whereas orbitofrontal activity in contingency unaware subjects might reflect uncertainty regarding the valence of the two contexts. In a next step we used a method termed "dynamical connectivity regression (DCR)", to estimate partial correlation matrices of 23 anatomical regions of interest for both contingency groups. The DCR analyses yielded a more densely interconnected network in the contingency aware group with more connections of the ventral striatum to frontal regions in these subjects compared to contingency unaware subjects. Our results indicate that functional connections between striatum and frontal cortex might be crucial for contingency learning.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: DFG, SFB 779

Hamburg state cluster of excellence (neurodapt!)

Title: The human substantia nigra dissociates between anticipation of social reward and punishment: evidence from human intracranial recordings

Authors: *N. BUNZECK[1,2], E. M. BAUCH[1], H. HINRICHS[3], F. C. SCHMITT[3], J. VOGES[3], H.-J. HEINZE[3,4], T. ZAEHLE[3,4]
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Abstract: It has repeatedly been shown that the dopaminergic midbrain, specifically the substantia nigra / ventral tegmental area (SN/VTA), responds to reward and cues that predict a rewarding outcome. However, there is increasing research, predominantly in animals, that questions its reward-specific role by showing that dopaminergic SN/VTA neurons also code salient non-rewarding and aversive events. Up to now, it is largely unknown how the SN/VTA differentiates between appetitive and aversive events. To elucidate this question, we tested four Parkinson patients with intracranial recordings in the SN/VTA during a social incentive delay task. Here, three visual cues predicted social reward (photo of a human indicating approval), social punishment (human indicating disapproval) or neutral outcome (human with neutral expression), respectively. Patients were motivated to react as quickly as possible to a dot presented between cue and social feedback to gain social reward or avoid social punishment. As a main finding, we can show differences between the anticipation of reward vs. punishment-based feedback in the local field potentials (LFPs) at ~300ms after cue onset and in oscillatory power in the theta (4-8 Hz) and high beta (18-30 Hz) frequency range. More specifically, theta power decreased to cues that predicted social reward relatively to aversive feedback; beta power showed the opposite effect. Together, our findings show compelling evidence that the human SN/VTA signals the anticipation of appetitive and aversive social information by distinct neural mechanisms. From a broader perspective, this further underlines a rather general role of the dopaminergic midbrain in processing motivationally relevant information.

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Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH097085

Title: How expectations shape aversive learning

Authors: *L. Y. ATLAS[1,4], C. SANDMAN[2], E. A. PHELPS[3]
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Abstract: Expectations profoundly influence perception and affective experience. Associative learning, or classical conditioning, has been described as the process by which an organism develops expectations about stimuli in its environment (Sutton and Barto, 1981). Consistent with this account, many studies incorporate expectancy ratings in human studies of Pavlovian aversive learning. It is thought that these ratings simply increase engagement with the task. However, reporting expectations might shift attention and induce higher-order beliefs that can in turn modulate associative learning. To our knowledge, no studies have formally measured whether expectations influence dynamic associative learning. The current study investigated whether expectations shape classical conditioning. Participants (n=40) were randomly assigned to either an Expectancy Group or a Passive Group. The Expectancy Group rated the probability of upcoming shock on every trial, whereas the Passive Group made no ratings. Both groups of participants underwent the same Pavlovian aversive learning paradigm wherein one image (the CS+) was paired with a shock on 50% of trials, while a second image (the CS-) was never paired with a shock. Halfway through the task, contingencies reversed. Skin conductance response (SCR) was measured to assess conditioning, and we assessed responses using both standard categorical analyses as well as quantitative learning models designed to assess participants' learning rates. Rating expectations increased SCRs overall ($p < 0.05$) but did not enhance conditioning, as measured by differential responses to the CS+ relative to the CS-. Instead, participants in the Expectancy Group exhibited slower learning rates than the Passive Group ($p < 0.05$), and were less likely to update differential responses when contingencies reversed. Within the Expectancy Group, expectancy ratings and SCR were highly correlated from trial to trial ($p <0.001$). These results suggest that rather than enhancing simple associations, making expectations explicit draws attention to higher order beliefs and rules that in turn reduce associative learning.

Disclosures: L.Y. Atlas: None. **C. Sandman:** None. **E.A. Phelps:** None.

Poster

457. Human Learning: Feedback, Reinforcement, and Reward

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Topic: F.01. Human Cognition and Behavior

Support: NSERC Discovery Grant

Title: The impact of alcohol hangover on reward processing within medial-frontal cortex

Authors: *A. D. HOWSE, C. D. HASSALL, O. E. KRIGOLSON
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Abstract: There is strong evidence for a reward processing system within the human medial-frontal cortex that plays a key role in reinforcement learning (Krigolson et al., 2014; Holroyd and Coles, 2002). Indeed, over the past two decades studies using electroencephalography have provided key evidence that the neural responses evoked by rewards and punishments respond in a pattern that would be predicted by computational theory (e.g., Sutton and Barto, 1998). Having said that, a lot remains unclear about the factors that influence the functional efficacy of the medial-frontal learning system. For example, behavioural evidence suggests that alcohol hangover impacts motor control and cognitive functioning (Cherpitel et al., 1998; Verster, 2007) and thus alcohol hangover may be one of the factors that would impair the medial-frontal system. In the present study, we aimed to provide the first electroencephalographic evidence of an impairment to the reward processing system of the human medial fontal cortex during alcohol hangover. Participants in our study completed a learnable gambling task while electroencephalographic data were recorded. In line with previous work, we found that the feedback error-related negativity (fERN; Miltner et al., 1997), a component of the human event-related brain potential (ERP), was elicited by wins and losses during the gambling paradigm. Further, and importantly, a reduction in fERN amplitude was observed for hangover relative to control participants. Additionally, we also observed a moderate correlation between fERN magnitude and hangover severity. Our data indicate the magnitude of impairment to the medial-frontal system increases with hangover severity. The results of the present study are important for public education and safety, as an impairment of the reward processing system within the medial-frontal cortex may implicate individuals' ability to execute corrective behaviours—processes necessary for successfully executing potentially dangerous tasks such as driving a motor vehicle.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Reward uncertainty enhances declarative memory encoding at long but not short latencies after cue presentation

Authors: *J. K. STANEK[1], N. J. CLEMENT[2], R. A. ADCOCK[3]
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Abstract: While it has been shown that the anticipation of reward modulates hippocampal memory encoding, it is still unclear how states of uncertainty about upcoming possible rewards influence such memory formation. At short latencies following presentation, probabilistic cues about future rewards stimulate phasic dopamine activity. As the probability of reward increases, so does phasic dopamine activity in the ventral tegmental area (VTA). However, at longer latencies following probabilistic cue presentation, sustained ramping dopamine signals in the VTA scale with the amount of uncertainty about reward, rather than the reward likelihood. Since previous work has claimed that both phasic (transient) and tonic (sustained) dopamine positively modulates hippocampal encoding, we were interested in whether declarative memory encoding would match the patterns of dopamine activity in response to reward probability. We therefore designed a study during which participants saw one of three pre-learned cues on each trial - 100%, 50%, or 0% predictive of an upcoming monetary reward. Everyday objects were presented at short latencies after cue onset (400ms) or long latencies after cue onset (3-3.6s), in the latter case just before reward feedback. We then tested recognition memory performance for the objects after a 24-hour delay. We found that of the objects presented at longer latencies, those encoded during states of reward uncertainty were significantly better remembered. While overall memory was comparable at short latencies, there was no such benefit of reward uncertainty. Additionally, memory performance was equivalent for 100% and 0% predictive trials at short latencies, suggesting that transient dopamine tracking reward likelihood did not benefit memory on these trials. These results are consistent with the theory that sustained but not transient dopamine signals in response to reward uncertainty enhance the mnemonic scope and strength of external environmental representations.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Hormonal and cognitive assessment of spatial ability and performance in engineering examination activities

Authors: *I. VILLANUEVA[1], W. GOODRIDGE[1], N. J. A. WAN[2], M. M. VALLADARES[1], B. S. ROBINSON[2], K. JORDAN[2]
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Abstract: Spatial ability and performance, associated with success in engineering, is a competency believed to not be uniformly acquired with gender, age, or environment. In this study, we are interested in understanding from a hormonal and cognitive standpoint, how engineering students perform when asked to solve common statics problems representative of this competency. Pre- and post- hormonal/cognitive profiles along with exam data were collected to understand predominant factors (gender or environmental stress) in an individual's spatial performance during an examination session where each problem had an added level of difficulty. For hormonal profiles, saliva kits were employed before and after the examination. Synchronously, a 14-channel electroencephalograms (EEGs) was placed on top of the participants' scalps to understand cognitive function as they solved the static problems. For the EEGs, alpha and beta brain wave activation frequencies were collected. Greater alpha activation implicates neural efficiency (ease-of-processing) while beta activation relates to conscious processing (performance) during a cognitive task. Our hypothesis is that increasing the cognitive requirements for statics problems will result in higher hormone fluctuations that could correlate with an individual's spatial ability during examination. Preliminary studies on eight engineering students showed that for the exam session, males performed worse (M = 54%) compared to the female participants (M = 65%; t =1.86, p = 0.077). EEG data, indicated by a percent change from the baseline, demonstrated no differences in alpha activation (8-12Hz) although an increase in beta (12-30Hz) activation was found for the females (+62%) compared to males (-7%; z = 2.236, p = 0.036) suggesting higher cognitive processing by the females. For gonodal hormones, female participants showed marginally significant reductions of estradiol (p=0.058) and marginally significant increases of progesterone (p=0.08) and testosterone (p=0.057) compared to males, suggesting gender differences during examination. Adrenal hormones dehydroepiandrosterone sulfate and cortisol showed no significant differences due to gender (p=0.40 and p=0.42, respectively) implying that external stressors did not affect spatial performance. Together the results suggest that increasing the difficulty level in the statics problems resulted in higher cognitive processing for females that may have been enhanced by changes in gonodal hormone levels during examination. The data also points to possible divergent gender-related brain processes for spatial ability, meriting further study.

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Poster

458. Human Decision-Making: Social and Emotional Factors

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Program#/Poster#: 458.01/SS27

Topic: F.01. Human Cognition and Behavior

Title: Does awareness affect embodiment of physical warmth in promoting interpersonal warmth?

Authors: *J. A. PINEDA[1], H. KOPPANG[2]
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Abstract: Objectives: In human evaluations of social behaviors, one of the first impressions formed is whether someone is a warm person or not. This warmth dimension appears to underlie group stereotypes across different countries and cultures and has a role in how humans interact and work together. A study by Williams and Bargh (2008) showed that experiences of physical warmth increases feelings of interpersonal warmth without a person's awareness of this influence. However, awareness was not manipulated in this seminal study. To examine the role of awareness, we had subjects in a similar condition to the original study and one in which participants were made aware of the goal. Methods: Sixty participants were pseudorandomly included in an Aware or Unaware condition. Participants were greeted in the kitchen of the Cognitive Science Department and asked to fill out a consent form as well as the International Personality Item Pool-Interpersonal Circumplex scale. Participants were then led to the main lab. On the way, which took approximately 3-4 minutes, they were asked to hold either a cup of warm tea or ice-cold water to help while the experimenter filled out a series of questions. Once in the lab, some participants read the abstract from the Williams and Bargh (2008) study, while others read a cover story - an abstract about consumerism. Participants were then asked to fill out a Personality Impression Scale about an imaginary individual (either a male or female) and a Car Rating Scale about automobiles. Finally, they were debriefed as to their insight into the purpose of the experiment including the role of the hot tea/ice water. Results: •Temperature of the cup had an effect on assessment of personality but not on assessment of cars. Warm tea produced more positive assessments compared to cold water. Cold produced the same trend but greater

variance, i.e. increased standard deviation compared to warm. •Awareness had no effect on judgment of personality but it had highly significant effects on judgment of cars. Aware individuals judged cars very positively compared to unaware individuals. The trend was similar for judging personality but not significant. •The difference between judging a male and a female approached significance. Females were judged more positively than males. •There was a correlation between dominant personality and car ratings. Conclusions: As reported by Williams and Bargh (2008), physical warmth from a warm cup of tea had a greater effect on assessment of another individual's personality than on an assessment of an object, such as a car. However, awareness of the conditions of the experiment had no effect on judging a person but did affect judgment of the car.

Disclosures: J.A. Pineda: None. **H. Koppang:** None.

Poster

458. Human Decision-Making: Social and Emotional Factors

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Topic: F.01. Human Cognition and Behavior

Support: NAVSEA

IARPA

Title: A multi-modal examination of the biophysical signals of interpersonal trust in an interative, computer-based gaming environment

Authors: *A. J. HAUFLER, A. FIRPI, M. GOFORTH, A. GREENBERG, M. HAPPEL, A. LEE, J. MILLER, M. OSORNO, D. RAGER, D. ROLLEND, J. SPITALETTA, R. VOGELSTEIN
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Abstract: Trust is both the willingness to be vulnerable to another person coupled with confident expectations of their behavior (Borum, 2010). It is a critical feature of our interpersonal interactions and is essential to the development of cooperative and pro-social behaviors. Based on an interpretation of the Social Exchange Model (SEM) in which specific, directional biophysical responses were postulated to be associated with trust and no-trust behaviors, the purpose of the present study was to examine the biological correlates of trust and assess the

ability to predict trust through neural, physiological, behavioral and/or biochemical assays. Trust was tested in friend/stranger pairings (n=80) and in dyads, introduced virtually via guided web chats prior to game play (N=80). 160 male and females aged 21-50 yrs played an interactive, iterative, computer-based game in which trust and trustworthiness were operationalized as integrity (promises made/kept), benevolence (amount of investment dollars), and competency (dollars earned from maze task performance). Electroencephalography (EEG), electrocardiography (ECG), and electro-dermal activity (EDA) were continuously recorded during gameplay and co-registered to biological and performance responses to specific events during gameplay. In addition, six blood samples were obtained from all participants, at key points in the game to assess the neuroendocrine (oxytocin and cortisol) response to the trust exchanges. Examination of the performance metadata revealed that there were more trust (55.6%) than mistrust (15%) decisions and more trustworthy (63.1%) than untrustworthy (36.9%) actions observed. Biophysical data were subjected to 6 x 2 x 4 (Measures: Biophysical metrics x Epochs: pre- and post-trust decision x Group: Friend, Stranger, Virtual Interaction, Pooled) ANOVAs to test the SEM predictions for biophysical signals. Low frequency HRV ($p=4.93E-03$) increased while R-R interval ($p=1.25E-09$) and SCL ($p=7.78E-10$) decreased in players just prior to making a trust decision. High frequency HRV ($p= 6.36E-03$), R-R interval ($p=5.99E-08$), and SCL ($p=5.79E-04$) decreased while low/high HRV ratio increased ($p=1.42E-03$) in players following a trust decision. SCL decreased prior to and following a no-trust decision ($p=4.03E-11$ and $p=7.98E-09$, respectively). Contrary to expectations, no change in the concentration of oxytocin or cortisol was revealed as associated with trust or no-trust decisions in the pooled data. In conclusion, cardiovascular and electro-dermal signals were reliably correlated with decisions to trust but the direction of signal change was inconsistent with predictions.

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Poster

458. Human Decision-Making: Social and Emotional Factors

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Topic: F.01. Human Cognition and Behavior

Support: Center for the Study of Ethical Development

Title: The impact of transcranial direct current stimulation on moral decision-making

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Abstract: Research investigating the neuroanatomy of moral judgments have used imagining techniques to identify structures that are particularly active when the individual makes moral judgments. The proposed presentation differs from this strategy in two ways. First, we focus on tDCS using the established structures as starting points and test whether manipulating neuronal excitability of these locations will alter moral judgment characteristics. Second we used established measures from moral psychology (Social Domain Theory -SDT) as dependent variables. According to SDT, concerns about fairness, justice, rights and welfare comprise the moral domain which is distinct from the social conventional domain and associated concerns about social norms, traditions, and authority. We also use items that are considered mixed domain, in which the described situation has elements of both types of rule violations (e.g., wearing a bathing suit to a funeral). Focusing on the Medial frontal gyrus (Brodmann area 9/10, FP2 10-20 system) and the posterior cingulate cortex (Brodmann area 31, P4 10-20 system) participants were placed in one of three conditions: 1: Anode on FP2 (positive electrode), Cathode P4 (negative electrode); 2: Anode P4, Cathode FP2; 3: Sham (same placement as in condition 1). Participants were stimulated for 20 minutes at 2 milliamps. Following the stimulation phase and using traditional social domain practices, participants were asked to consider 21 items (7 moral, conventional and mixed), in terms of the seriousness of the violation, and the degree these rating vary by context (other community and other country) on 5-point likert scales. Items for each domain (and mixed items) were summed resulting in three summary scores. Using a 2-way repeated measures ANOVA (condition as the between and domain as the within subjects factor), results indicate the expected main effect for domain (moral violations viewed as most serious, followed by mixed and conventional items). Importantly, a statistically significant effect for condition (Condition 1 resulted in more serious ratings than the sham condition with condition 2 falling in between). A statistically significant interaction between conditions and domain was also noted. Inspection of the means suggests that the effect associated with conditions was strongest for the conventional items, whereas the moral item rating remained unchanged. These data support the role of the two regions in judging the seriousness of rule violations particularly in the conventional domain. Additionally these findings are significant in providing additional support for the distinction between moral and conventional domains.

Disclosures: S. Thoma: None. **R. Houser:** None. **E. O'Conner:** None.

Poster

458. Human Decision-Making: Social and Emotional Factors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

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NUS grant WBS R-581-000-123-133

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Title: Moral judgment modulation by disgust priming through altered fronto-temporal functional connectivity

Authors: O. MULLETTE-GILLMAN[1], Y. A. KURNIANINGISH[1], H. ONG[1], F.-C. QUEVENCO[2], K. KWOK[2], *J. Z. LIM[2]
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Abstract: We investigated the neural mechanisms of emotional modulation of moral judgments utilizing a paradigm known to have robust priming effects (Ong et al., 2014), within this, disgust priming alters the moral acceptability of actions as a bi-directional function of individual sensitivity to disgust. Here, we show that the change in behaviorally-rated acceptability covaries with individual differences in neural activations in the dorsomedial prefrontal cortex (dmPFC). Through functional connectivity analyses, we show that the emotional prime results in significantly reduced connectivity between dmPFC and bilateral temporal-parietal junction (TPJ). Our findings suggest a model through which moral decision making is the product of the integration of deliberative reasoning with social and emotional information. Participants (N=19) underwent fMRI scanning while judging the acceptability of actions in a set of moral dilemmas. Dilemmas always involved harming one person so as to save the lives of multiple others. Prior to each trial, subjects were subliminally primed with a face displaying either a disgusted or neutral expression. Post-scan, subjects completed the Disgust Sensitivity (DS) Scale - Revised (Olatunji et al., 2007). Pooling these subjects with our prior participants (N=74) (Ong et al., 2014), revealed an overall significant function between the priming effect and DS ($r = .35$, $p < .01$), although the relationship did not reach significance in the smaller neuroimaging sample alone. This function revealed bi-directional modulation of moral acceptability by presentation of disgust stimuli - high sensitivity to disgust enhances moral acceptability while low sensitivity reduces it. No significant activation differences were found between the disgust and neutral conditions during the priming or decision phases. Using the behavioral change in acceptability due to priming (disgust - neutral) as a between-subject covariate, we found significant modulation in the dmPFC, ventromedial prefrontal cortex, and right middle temporal cortex. PPI analyses of the dmPFC ROI at the time of priming revealed that the dmPFC ROI was

functionally connected with bilateral TPJ during the neutral, but not the disgust prime, with the difference in weights between these conditions correlated with DS scores ($r = .55$; $p < .05$). Examining the specificity of this effect, the dmPFC was also functionally connected to one other cluster, including the lateral occipital cortex, with connectivity unaltered by the emotionality of the subliminal faces.

Disclosures: O. Mullette-Gillman: None. **J.Z. Lim:** None. **Y.A. Kurnianingish:** None. **H. Ong:** None. **F. Quevenco:** None. **K. Kwok:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NRF-2012R1A2A2A04047239

Title: A fear of sticking out: Role of BNST in conforming to the majority

Authors: *H. JUNG[1,2], H. KIM[1,2]
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Abstract: People often change the course of their behavior under social pressures, as to blend in with the group. This phenomenon, known as social conformity, is mainly associated with fear of being different from group; yet, the understanding of its neural mechanism is limited. In this study, 33 female participants performed a preference rating/choice task on typefaces for three consecutive experiments, two weeks apart. Test 1 and 3 were designed to track behavioral patterns in the absence of social influence whereas Test 2, a two-item choice task in a fMRI scanner, aimed to examine neural processes modulated by social influence. Social influence manipulation was done by placing a tag on one of the two items, ostensibly informed as the "choice of the majority," which was decorrelated with individual's preference across trials. Participants chose an option tagged incongruently with their own preferences more often during Test 2 than Test 3. FMRI data showed greater activity in the lateral orbitofrontal cortex (lOFC) when participants chose incongruently versus congruently tagged options, and the opposite contrast revealed the ventromedial prefrontal cortex (vmPFC). In addition, when participants chose against the group versus followed the choice of majority for incongruently tagged items, greater activity was observed in the Bed Nucleus of Stria Terminalis (BNST), which has been reported as the neural sector of 'threat monitoring.' Furthermore, as we were interested in the

two paths that conformity may take in the absence of social influence, we analyzed trials of persistently choosing the conformed option ("internalized conformity") versus those that did not ("transient conformity") during Test 3. Compared to transient conformity trials, the caudate nucleus activated during trials of the internalized conformity trials while the opposite contrast showed activations in the medial prefrontal cortex (mPFC). In summary, the present study provides a more sophisticated neural model whereby social pressures influence our decisions to conform to the majority. It is particularly noteworthy that threat signals arise in the BNST when a mismatch occurs between the individual and a group. When this threat signal triggers lOFC, prepotent individual preference encoded by vmPFC can be overridden, leading to social conformity.

Disclosures: H. Jung: None. **H. Kim:** None.

Poster

458. Human Decision-Making: Social and Emotional Factors

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Topic: F.01. Human Cognition and Behavior

Support: The MacArthur Foundation Research Network on Law and Neuroscience

Title: Parsing the neural components underlying third-party punishment decision-making

Authors: *M. GINTHER[1], O. D. JONES[2], R. MAROIS[3]
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Abstract: There is a substantial amount of concordance in punishment assessments made by third parties. However, relatively little is known about the shared neurobiological mechanisms that this concordance suggests exists. Buckholtz & Marois (2012) have proposed a model positing that affective processes, encoded in a cortico-limbic circuit, and socio-evaluative processes, associated with the temporoparietal junction (TPJ), are integrated by the dorsolateral prefrontal cortex (DLPFC) to assign appropriate punishments. In the present study we use functional MRI to parse the primary components that contribute to these third-party punishment decisions: the actor's mental state and the resulting harm. We employed a novel experimental design that allowed us to distinguish the neural processes that are recruited to evaluate the mental state (i.e., intentions) of the actor from those recruited to evaluate the harm that results from the actor's conduct. Further, our design allowed us to distinguish the brain regions engaged by each

of these evaluative processes from those involved in the integration of these distinct components into a punishment decision. Specifically, we parametrically manipulated in textual scenarios both the actor's mental state (from blameless to negligent to reckless to purposeful) and the resulting harm (from no injury to substantial injuries to life-altering injuries to death) while presenting each of these evaluative stages as well as the decisional stages sequentially, thereby rendering them temporally resolvable with fMRI. The behavioral data (n=23) demonstrated a strong interaction between the levels of mental state and the harm on individuals' punishment ratings, consistent with theoretical models of third-party punishment. The functional imaging results revealed that, compared to harm evaluations, mental state evaluations are associated with heightened TPJ activity. Conversely, as compared to mental state evaluations, harm evaluations are associated with heightened activity in the posterior insular cortex. Greater severity of the harm or culpability of the mental state both correlated with increased activity in the amygdala. The interactive effect of harm and mental state on punishment, however, was reflected in right DLPFC activity. Finally, we found that relative to the evaluative stages, analysis of the punishment decision phase of the task revealed increased activity in right DLPFC as well. Together, these results support and advance the Buckholtz and Marois (2012) model of third-party punishment decision-making.

Disclosures: **M. Ginther:** None. **O.D. Jones:** None. **R. Marois:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: ONR MURI Award No.: N00014-10-1-0072

Title: Design of a virtual reality hyperscanning environment

Authors: ***J. SNIDER**[1,2], J. TREES[1,2], M. FALAHPOUR[2], N. GUO[2], K. LU[2], D. C. JOHNSON[2,3], H. POIZNER[1,2], T. LIU[2,4]
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Abstract: Humans are inherently social animals whose interpersonal interactions are at least as important as their individual ones. Thus, the human brain must not only represent its own

environment and carry out its own actions, but also incorporate or model how other individuals are representing the environment, and, in particular, what actions they will take. Such models are recursively complex and come under the rubric of the 'theory of mind' that philosophers have discussed since at least Descartes' time. Only recently have neuroimaging techniques progressed to the point that experimental observations of the theory of mind at work are becoming possible. Hyperscanning is one such emerging technique devoted to studying brain activity of multiple, interacting subjects simultaneously. Here we present a system that extends hyperscanning techniques to MRI recordings from two subjects interacting within a virtual environment. The primary goal is to move beyond abstract interactions, like talking on the phone, to more realistic person-to-person interactions, all the while maintaining the ability to obtain high quality fMRI measures of brain activity. The virtual reality (VR) environment Virtual Battlespace 2 (VBS2) is used for military training and includes realistic game physics and avatar-avatar interaction. For participant control of VBS2 avatars, we modified Play Station 3 game controllers for the MRI environment by removing extraneous metal components and replacing necessary ones with 3D printed plastic versions. Electromagnetic shielding of the controller entailed connecting it to a through panel with shielded wire and spraying a silver-copper conductive coating on its inside cover. The controller shielding was grounded to the MRI room shield to effectively isolate it from the MRI, even when it was physically within the bore of the magnet. This system successfully prevented interference both to the MRI from the controller and vice versa. VBS2 itself programmatically initiated the fMRI scan at a known time within the VR simulation. The virtual world was represented to the participants using a projector screen. The end result was an immersive VR experience for two interacting subjects within the MRI. Most importantly, pilot fMRI data were artifact free. Preliminary runs, just exploring the VR, revealed differences in brain response to mutual eye contact, and joint spatial ICA of the two brains resulted primarily in ICs that were localized to one of the two brains. With this hyperscanning system in place, we will now be able to apply powerful MRI neuroimaging techniques to study realistic interactions between people performing tasks in which cooperation is essential.

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Poster

458. Human Decision-Making: Social and Emotional Factors

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Topic: F.01. Human Cognition and Behavior

Support: NRF-2012R1A2A2A04047239

Title: The fairness model based on EEG hyperscanning for behaviors in competitive games

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Abstract: Understanding of the non-linguistic communication between two persons is important to explain the behaviors during social interaction. In the present study, the "chicken game" was used as a competitive game. Each of two participants controlled one of the two cars that rush toward each other and they were to decide whether to avoid or not. The reward or loss was determined according to their decision in the context of the opponent decision. To collect the EEG data, a hyperscanning method was used. EEG signals were measured simultaneously for two participants during the experiment. There were various behavioral patterns obtained, including four distinct patterns: avoidance in turn pattern, mutual rush pattern, mutual avoidance pattern, and unfair pattern. Some of those patterns were inconsistent with the predictions based on typical reinforcement learning models, such as the Rescorla-Wagner model, which assume the rational pursuit of reward. To explain those behaviors, we developed the "fairness model" that predicts the behavior through the relative sense of fairness of each participant. The fairness model explains all the distinct patterns well. Furthermore, the hyperscanning method enabled the construction of a modified fairness model using post-trial theta band (4-7Hz) power at frontal areas as the input. This EEG-based model made even better predictions of behavior than the behavior-based model using behavior itself as the input. These outcomes are consistent with Cohen et al.'s (2007) claims suggesting that the theta band power was related to the outcome calculation after the trial. The result indicates that non-linguistic processing of social interaction can be better explained by continuous neural signals than the discrete behavior. In addition, we found the increasing gamma band (25-50Hz) power at FC2 channel before avoidance compared to rush. The changes of gamma band were more robust for the people with fair behaviors. The increasing gamma band power possibly reflects the signals from DLPFC when inhibiting the urge to rush and it seems to be closely related to the sense of fairness of the participants. The behavioral results from the chicken game is well explained by post-trial outcome calculation related to the theta band power and pre-trial action inhibition related to the gamma band power. Those frequency bands were both related to the sense of fairness, implying that the fairness plays a critical role in the competitive social interaction. Sharing of the sense of fairness is one of the important forms of non-linguistic social communication that determines social behaviors.

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Poster

458. Human Decision-Making: Social and Emotional Factors

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Program#/Poster#: 458.09/SS35

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26120732

Title: How is reward of others added to make one's own decisions in neural mechanisms?

Authors: *H. FUKUDA[1,5], S. SUZUKI[1,6,7], N. MA[1], N. HARASAWA[1], K. UENO[2], J. L. GARDNER[3], N. ICHINOHE[8], M. HARUNO[9], K. CHENG[2,4], H. NAKAHARA[1]
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Abstract: Humans make decisions guided by the expectation of their own rewards, but also often influenced by their decisions' consequence to rewards of others. A fundamental question in social cognition is what the underlying neural mechanisms are. This study addressed the issue, devising a novel task and combining human fMRI with computational modeling (model-based analysis). Our task is composed of three types of trials: Control, Charity and Bonus. Control trials served as baseline, i.e., value-based decision-making to choose one of two options (for self reward), whereas Charity and Bonus trials involved an additional reward to either option: charity and bonus (to others and the self, respectively). Thus, this task allowed us to measure quantitatively the influence of others' outcomes on self-decisions, relative to the influence of one's own additional outcome. Behaviorally, we found that a majority of subjects are influenced by the additional, charity reward. The influence is well characterized as an addendum to the original self-reward value, still weaker than that by the same face value of bonus. Analyzing BOLD signals, we found that the signals in temporoparietal junction are significantly correlated with charity value, but not with bonus value. The signals in ventromedial prefrontal cortex and frontal pole are significantly correlated with decision value in Control trials. In Chairty and Bonus trials, i.e., when the decision value is summation of the original value with charity and bonus value, the signals in the same region indicated distinct modulations. The results together demonstrate that neural signals for reward to others are different from those for additional reward to the self in both of the first and final stages (encoding each additional value and total final

value). This suggests a fundamental distinction in reward of the self and others for making decisions.

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Poster

458. Human Decision-Making: Social and Emotional Factors

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Topic: F.01. Human Cognition and Behavior

Support: NWO; HCMI 10-19

Title: Shifting the balance: Interfering with motor inhibition and reactive aggression by inducing fronto-cortical asymmetry using transcranial direct current stimulation (tDCS)

Authors: ***F. DAMBACHER**[1], T. SCHUHMANN[1], J. LOBBESTAEL[2], A. ARNTZ[2], S. BRUGMANN[2], A. T. SACK[1]
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Abstract: It has been proposed that prefrontal regions within the right hemisphere are predominantly involved in avoidance motivation, whereas such regions within the left hemisphere are mainly linked to approach motivation. According to this concept, cortical asymmetry resulting in right-hemispheric fronto-cortical dominance should lead to improved inhibitory capacities, no impulsivity, and less aggressive behavior, whereas cortical asymmetry resulting in left-hemispheric fronto-cortical dominance should lead to deficient inhibitory capacities, impulsivity, and more aggressive behavior. Here, we used 1.5mA tDCS applied to inferior frontal cortex to either induce right, left, or no (sham condition) fronto-cortical asymmetry in sixty healthy, young males in a between-subject design. During stimulation, inhibitory capacities were measured by a simple motor inhibition task (go-/nogo task). Reactive and proactive aggression was evaluated by a version of the Taylor aggression paradigm in which participants could administer uncomfortable noise punishment to a putative opponent whenever they won during a competitive reaction time game. We controlled for the initial appraisal of the opponent, trait aggression, and the experience of side effects caused by the brain stimulation.

Data collection is still in progress. Based on the sample currently available (n=30), the induction of right- or left-hemispheric dominance compared to sham stimulation had no influence on neither motor response inhibition (univariate analysis of variance $F(2,87)=1.315$, $p=.285$) nor aggressive behavior (univariate analysis of variance $F(2,87)=.781$, $p=.468$). This null finding might result from the lack of sufficient statistical power due to the rather small sample size on which this preliminary analysis is based. Therefore, these preliminary results might change once the a priori calculated optimal sample size has been tested. Alternatively, i.e. in case statistical evidence falsifying the null hypothesis of our model will remain low, this study may also indicate that - within the framework of the here presented experimental design - theories on cortical asymmetry might not easily apply to non-invasive brain stimulation methods. It might be that more complex models have to be exerted in order to induce actual behavioral effects by shifting fronto-cortical balance.

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Poster

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Support: NIH Grant AG017586

Title: A social-interactive approach to strategic reasoning in behavioral variant Frontotemporal Dementia

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Abstract: Introduction: Behavioral variant frontotemporal dementia (bvFTD) is a neurodegenerative disease that leads to executive and social deficits associated with atrophy in frontal and temporal cortex (Rascovsky et al, 2011). Previous studies have associated bvFTD social impairments with decision-making limitations (McMillan et al, 2012) but, to our knowledge, no prior work in bvFTD has empirically investigated reasoning in a social-

interactive context. In this study we report an interactive game in which participants played against a competitor in an effort to maximize a reward. We hypothesized that bvFTD would have difficulties with strategic reasoning in an interactive context with relatively preserved reasoning in the absence of social interaction. **Methods:** 16 bvFTD patients and 16 demographically-matched healthy controls were presented with a game in which a marble can drop in an array following alternative paths. Every path leads to a different amount of points and the goal of the game is to earn the most points possible. Participants can guide the marble opening a door at 2 levels. In a Control Game participants played alone and chose which door to open at both levels in order to maximize their points, while in an Interactive Game participants set the door at the first level and a confederate then sets the door at the second level. Here, patients must anticipate the confederate's move and develop an alternate strategy to maximize possible points. **Results:** bvFTD performance in setting the door at the first level were significantly worse in the Interactive Game compared to the Control Game ($p<.05$), while controls performed almost at ceiling in both tasks ($p>.5$). We used a voxel-based morphometry analysis (VBM) in a subgroup of 13 bvFTD with available structural scans to relate gray matter atrophy to a d-prime score of performance in the Interactive Game that controlled for errors in the Control Game. Errors in the Interactive game were related to atrophy in right inferior frontal gyrus (IFG, BA47) and right insula ($p<.01$; uncorrected). **Conclusions:** bvFTD have limited strategic reasoning when they must take into account a competitor's performance in a simple game. VBM related strategic limitations to atrophy in areas previously linked to risk-assessment (Tom et al, 2007). These findings support the critical role of right IFG and insula in social-interactive aspects of strategic reasoning. We also highlight the importance of patient-based studies for advancing interactive studies in social neuroscience.

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Poster

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Support: Pro-Vice-Chancellor's Fund, University of Reading PhD Studentship

Title: Social congruence influences the evaluation of decision outcomes via the ventromedial prefrontal cortex, insula and anterior cingulate

Authors: A. VARJACIC, T. JOHNSTONE, *J. D. SADDY, A. CHRISTAKOU
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Abstract: People make decisions in the context of others. Neuroimaging evidence suggests that a network of brain regions scales the value of decision outcomes according to the social context in which decisions are made. For example, evidence suggests that the ventral striatum, ventromedial prefrontal cortex (vmPFC) and insula track the degree of social alignment during evaluation of outcomes. It has been argued that the vmPFC holds a general context representation; however, there are also suggestions that the vmPFC encodes the quality of the social context. In this study, we investigated whether the vmPFC discriminates between different social contexts. Further, we aimed to identify the brain areas that mediate the moment-to-moment integration of social context and decision evaluation. We designed a task in which participants experienced wins and losses in light of varying levels of agreement with the choices of other study participants ("social congruence" condition). Social congruence was conveyed through a graphic before outcome presentation. By contrast, in the control variant of the task, we used the same graphic to inform participants of the probability of obtaining a favourable outcome ("probability" condition). Male participants (N=20 per condition) underwent functional magnetic resonance imaging whilst performing the task. Behaviourally, we found that the level of signalled social congruence interacted with the nature of the experienced outcome, with losses following low congruence leading to deviations in choice behaviour. This effect was absent in the control probability condition. At the neural level, the vmPFC differentiated between high and low feedback in the social congruence, but not in the probability condition. Using a parametric modulator approach, we found that decreasing social congruence was correlated with increased activation in insula and dorsal anterior cingulate (dACC) during wins and in vmPFC during losses. Between-condition comparison of the modulated maps revealed that context-dependent processing of wins recruited overlapping neural circuitry in both social and probability contexts. By contrast, evaluation of losses was more strongly modulated in anterior insula in the social compared to the probability context. Our findings extend our understanding of the role of the insula and dACC in mediating the influence of contextual information on the evaluation of outcomes, and lend support to the idea that the insula is critical for guiding behavioural adaptation via increased sensitivity to incongruent social feedback. Finally, they suggest a specific role for the vmPFC in representing social context.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Grant from American Legacy Foundation

Title: Young adult smokers' reactions to graphic warning labels and tobacco advertising

Authors: *N. M. GALLAGHER[1], R. S. NIAURA[3], K. P. TERCYAK[4], D. E. VALLONE[3], D. MAYS[4], A. GREEN[2]
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Abstract: Considerable existing research supports the efficacy of mass media anti-smoking campaigns in altering tobacco-related knowledge and attitudes, as well as helping to prevent smoking and promote quitting. However, the mechanisms by which this happen remain unclear. This project aimed to explore the reactions of young adult smokers to cigarette and e-cigarette advertising, as well as the impact of graphic warning labels. This project recruited a sample of young adult smokers (M=22.04 years, SD=3.37, 74% male). All participants were current smokers with no history of brain damage or disorder. Participants rated a series of advertisements - combustible cigarettes, e-cigarettes, and control advertisements on a scale of 1-4 to indicate how much each advertisement made them want to smoke. A separate task investigated the effect of graphic visual cigarette warning labels on smokers' desire to quit smoking. Participants rated images of cigarette packs (1-4) on how much the images made them want to quit smoking. These images of cigarette packs varied by type of warning label - graphic visual, control visual, and text. The mean ratings of desire to smoke in response to tobacco (M=2.32, SD=0.72) and e-cigarette advertisements (M=2.15, SD=0.45) were not significantly different (t(15)=1.04,p=0.313). However, the mean rating of control advertisements (M=2.15, SD=0.36) was significantly lower than both (t(15)=5.44, p=0.00; t(15)=6.385, p=0.00). The mean response time to tobacco advertisements (M=1785.80ms, SD=457.30) was significantly faster than to e-cigarette advertisements (M=1878.96ms, SD=467.16; t(15)=-2.393, p=0.030). The mean rating of how much graphic visual warning labels made participants want to quit (M= 3.08, SD=0.82) was significantly higher than the mean rating for visual control (M=2.14, SD=0.87; t(18)=4.33, p=0.000) and text-based warning labels (M=2.06, SD=0.94; t(18)=3.909, p=0.001). There was no significant difference between the visual control warnings and the text

warnings. Moreover, the rating of how much a tobacco advertisement made an individual want to smoke and how much a visual warning label made that individual want to quit were significantly positively correlated ($r=0.70$, $n=13$, $p=0.007$). Taken together, these findings support the possibility that - while e-cigarettes are new and still unfamiliar - their marketing does not reduce the desire to smoke relative to tobacco marketing. They also demonstrate the efficacy of graphic warning labels for reducing the desire to smoke, particularly in individuals with whom tobacco advertising creates greater desire.

Disclosures: **N.M. Gallagher:** None. **R.S. Niaura:** None. **K.P. Tercyak:** None. **D.E. Vallone:** None. **D. Mays:** None. **A. Green:** None.

Poster

458. Human Decision-Making: Social and Emotional Factors

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 458.14/SS40

Topic: F.01. Human Cognition and Behavior

Support: Impact Award to NG

Wellcome Trust Career Development Fellowship to TS

Title: How lying becomes a habit

Authors: ***N. GARRETT**[1], S. C. LAZZARO[1], D. ARIELY[2], T. SHAROT[1]
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Abstract: A habit is a behavior that is acquired through previous repetition and tends to occur unconsciously. Here, we examine how past decisions to lie form a habit of acting dishonestly. Past research (Mazar, Amir & Ariely, 2008) has shown that individuals are somewhat reluctant to engage in lying because they like to think of themselves as "virtuous". As a result they will lie just enough to profit while still maintaining a positive self-image. We developed a two party game where lying could be partially dissociated from virtue; in one block lying benefited both parties and in another block it benefited only the self. Our findings show that when lying initially benefits both parties it increases linearly over time, forming a habit. Subsequently individuals lie more even when lying only benefits themselves. fMRI data reveal that under this circumstance the brain is less likely to monitor the amount of lying on a trial-by-trial basis. In contrast, if lying

initially benefits the self a habit is not formed and BOLD signal continues to track deviations from the truth. This suggests that environments which make lying readily justifiable make deception for the pursuit of selfish gain more likely and decreases the likelihood of self-monitoring.

Disclosures: **N. Garrett:** None. **S.C. Lazzaro:** None. **D. Ariely:** None. **T. Sharot:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.01/SS41

Topic: F.01. Human Cognition and Behavior

Support: Rockefeller Training Grant

Title: Neural representation of the preferences for environmental public goods using contingent valuation

Authors: ***M. KHAW**[1], D. A. GRAB[2], M. A. LIVERMORE[4], C. A. VOSSLER[5], P. W. GLIMCHER[3]
[1]Neural Sci., [2]Inst. for Policy Integrity, [3]Ctr. for Neural Sci., New York Univ., New York, NY; [4]Sch. of Law, Univ. of Virginia, Charlottesville, VA; [5]Dept. of Econ., Univ. of Tennessee - Knoxville, Knoxville, TN

Abstract: Environmental public goods - including national parks, clean air/water, and ecosystem services - provide substantial benefits on a global scale. Though these goods are not bought and sold in markets, they offer value to a large number of individuals both in current and future generations. The passive use values inherent in these goods are unique and have not been explored by neuroscience. The behavioral focus of this study is contingent valuation (CV), a stated preference approach that presents survey respondents with information on an issue and asks questions that help policy makers determine just how much citizens are willing to pay for that public good or policy. Stated preference approaches are frequently used to evaluate large-scale public projects, to estimate the economic consequences of environmental regulations, and in litigation over natural resource damages. There is ongoing debate over the validity of the CV procedure for environmental goods (whether it accurately describes citizens' willingness to pay), but it remains the most prevalent tool for eliciting these preferences. Progress in decision neuroscience over the last decade has enabled scientists to relate preferences to neural activity in

the domains of consumer goods, social/monetary rewards, and many others. Here, we test the hypothesis that neural signals in areas correlated with previously examined valuations (ventromedial prefrontal cortex and ventral striatum) also correlate with preferences concerning environmental public goods valued via CV. This is the first study to attempt to identify the neural representation of value for environmental public goods using CV. We scanned human subjects (N=30) while they passively viewed and valued environmental proposals and three classes of goods previously examined by neurobiologists. The same subjects later completed a CV 'payment card' procedure for the very same proposals. Outside the scanner (also interleaved), subjects were asked to choose between previously viewed consumer goods, bid on snack foods, and rate the desirability of activities of daily living. While our examination of these three classes of goods replicated previous findings, preferences between environmental proposals yielded no statistically significant correlations in any region of the brain. Thus, our procedure replicates known valuation correlations for three classes of goods. In contrast, the environmental public goods valued with the CV procedure did not yield neural correlates at comparable statistical thresholds. The results show that the preferences regarding environmental public goods elicited by the CV procedure differ from traditional valuations.

Disclosures: M. Khaw: None. **D.A. Grab:** None. **M.A. Livermore:** None. **C.A. Vossler:** None. **P.W. Glimcher:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.02/SS42

Topic: F.01. Human Cognition and Behavior

Support: NIH NS054775

Title: The value representation of collections of goods for losses is not gain-loss context dependent

Authors: *H.-K. CHUNG[1], A. TYMULA[3], P. GLIMCHER[2]
[1]Psychology, [2]Ctr. for Neural Sci., New York Univ., New York, NY; [3]Econ., Univ. of Sydney, Sydney, Australia

Abstract: In economics, there are many mathematical models to describe value representations (utilities) that can be applied to explain or predict people's choice across different decision

situations. One important decision situation is that of purchasing a “bundle”, which is a collection of goods. For example, one might buy a bundle of 2 boxes of milk, and 4 packages of Goldfish, instead of buying one kind of good. Traditionally, economists use the “indifference curve” to represent combinations of goods which an individual finds equally desirable. By presenting subjects with choices between many bundles, for instance bundle A (4 boxes of milk and 4 pieces of chocolate) or bundle B (5 boxes of milk and 3 pieces of chocolate), we can identify all the bundles which offer that chooser the same utility of level utility. One of the assumptions many models of indifference curves makes is that each of the goods in a bundle presents a decision-maker with a diminishing marginal utility as the number of that bundle element increases. It predicts that people are more willing to give up1 box of milk for 1 piece of chocolate when they already have a lot of milk, but less willing to give up1 box of milk when they have fewer boxes of milk. However, many Prospect Theoretic based models, which described subjects as risk seeking in losses, predict that in the domain of losses the opposite of this usual pattern should obtain. Here, we propose two hypotheses for indifference curves under losses. First, the classical theory predicts a concave function in the domain of losses, which is the product of diminishing marginal utility. Second, Prospect Theory and its reference point might be thought to predict convex curvature - oddly suggesting an increasing marginal utility for goods. Perhaps surprisingly, this fundamental prediction of Prospect-like theories has never been empirically tested at the behavioral or the neural level. We therefore measured indifference curves in an incentive compatible procedure for gains and losses while also characterizing individual choosers’ risk attitudes in the domains of gains and losses. Despite the fact that our subjects show clear evidence of risk-seeking in losses (a finding compatible with standard Prospect Theory), we found that the indifference curves are concave not only for gains but also for losses, a finding not compatible with many Prospect-like theories. As we turn to the neural implementation of choice this seems a critical distinction as it suggests that the increasing marginal utility under losses which is a core feature of Prospect Theory cannot be observed in the domain of consumer choice.

Disclosures: **H. Chung:** None. **P. Glimcher:** None. **A. Tymula:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.03/SS43

Topic: F.01. Human Cognition and Behavior

Support: A Visiting Scholar position at UC Berkeley.

Title: Neuroeconomics and revealed-preference theory as synergistic cornerstones in economics: Linking neural and choice data may enable a novel self-regulatory policy for preventing asset-price bubbles

Authors: *J. L. HARACZ[1], D. J. ACLAND[2]
[1]Dept. of Psychological & Brain Sci., Indiana Univ., Bloomington, IN; [2]Goldman Sch. of Publ. Policy, UC Berkeley, Berkeley, CA

Abstract: Asset-price bubbles challenge the explanatory and predictive power of standard economic theory, so neuroeconomic measures should be assessed for a capacity to improve the predictive power of the standard approach. This assessment objective is achieved by reviewing results from functional magnetic resonance imaging (fMRI) studies of lab asset-price bubbles and herding behavior (i.e., following others' decisions). In subjects exposed to replayed visual displays of lab-market bubbles, activations were found in the medial prefrontal cortex (mPFC), an area implicated in theory-of-mind mechanisms, possibly reflecting subjects' attempts to sense peers' intentions (De Martino et al., 2013). Another lab-market study showed displays based on historical records of Lehman Brothers stock prices (Ogawa et al., 2014). Exposure to the Lehman Brothers bubble activated subjects' inferior parietal lobule (IPL) and increased functional connectivity between dorsolateral PFC and IPL, possibly suggesting a future-oriented mental focus during the bubble. These studies may have limited external validity: fast-growing lab bubbles differ temporally from long-lasting real-world bubbles (e.g., the housing- and stock-market bubbles that rose and crashed during 2000-2008). Herding may be hypothesized to occur during these prolonged real-world bubbles, in which case fMRI evidence for the involvement of evolutionarily ancient brain areas (e.g., nucleus accumbens, amygdala, hippocampus) in various forms of herding, including that related to financial decision-making (Burke et al., 2010; Edelson et al., 2011; Zaki et al., 2011), could be informative for predicting bubbles. Crucially, the same choice (e.g., buying a stock) could be generated by herding-related neurocircuitry during bubbles, or by deliberative neocortical circuitry during non-bubble periods. Using functional near-infrared spectroscopy headband technology (Shimokawa et al., 2009), it may be possible to identify herding behavior and thus predict bubbles. We propose a field-experimental research program to test this hypothesis, as well as non-intrusive interventions to prevent or mitigate bubbles that could be implemented without government involvement. For example, traders could monitor an open-access aggregated data stream of processed brain activity, collected from consenting traders, for real-time signs of over-heated markets, enabling them to exit these markets and thereby prevent major bubbles voluntarily. In conclusion, a synergism between neuroeconomics and the standard economic approach may be useful for distinguishing bubble and non-bubble periods and intervening in bubbles.

Disclosures: J.L. Haracz: None. **D.J. Acland:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

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Program#/Poster#: 459.04/SS44

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant F31-NS086254

McDonnell Foundation Collaborative Action Award

Title: Changes in functional connectivity after ventromedial prefrontal cortex damage relate to emotion-based decision-making behavior

Authors: *M. J. SUTTERER[1], M. W. VOSS[2], J. BRUSS[1], T. SLADE[1], N. L. DENBURG[1], A. BECHARA[3], D. TRANEL[1]
[1]Dept. of Neurol., [2]Dept. of Psychology, Univ. of Iowa, Iowa City, IA; [3]Dept. of Psychology, USC, Los Angeles, CA

Abstract: Decades of careful study of patients with focal brain damage have established the importance of the ventromedial prefrontal cortex (vmPFC) in complex, real-world decision-making. The somatic marker hypothesis predicts that the vmPFC integrates information from other affective and somatosensory areas (including the amygdala, insula, and striatum) in order to effectively utilize emotional information during decision-making. Functional neuroimaging and neuropsychological studies have confirmed the involvement of these other areas in decision-making, but it is not well understood how these regions are functionally connected at rest, and how chronic reorganization following damage to the vmPFC might lead to changes in functional connectivity between these areas. The present study examined differences in resting-state functional connectivity and performance on decision-making tasks in patients with damage to the vmPFC, patients with damage outside the vmPFC, and normal comparison participants. Using a region-of-interest approach, we compared these populations on amygdala, anterior insula, and striatum connectivity, and on the relationship between resting-state connectivity and decision-making performance on the Iowa Gambling and Cups Tasks. Patients with vmPFC damage, relative to normal comparisons, had significantly lower left and right ventral striatum connectivity. In addition, we observed a trend toward vmPFC patients demonstrating greater connectivity between the left and right amygdala than normal comparisons. Whole-brain group difference analyses of seed maps demonstrated greater connectivity between the right amygdala and areas in the right insula and putamen for normal comparisons relative to vmPFC patients. In contrast, left and right insula seed maps demonstrated greater left hippocampus and posterior

thalamic connectivity for vmPFC patients compared to normal comparisons. In regard to behavior, there was evidence in normal comparisons, but not vmPFC patients, for left to right amygdala connectivity being positively correlated with performance on decision-making tasks, and left to right insula connectivity being negatively correlated with decision-making performance. This work highlights changes in functional connectivity in non-damaged limbic and reward areas following focal vmPFC damage, and suggests these changes in connectivity might contribute to well-characterized behavioral impairments in decision-making in these patients.

Disclosures: **M.J. Sutterer:** None. **M.W. Voss:** None. **J. Bruss:** None. **T. Slade:** None. **N.L. Denburg:** None. **A. Bechara:** None. **D. Tranel:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.05/SS45

Topic: F.01. Human Cognition and Behavior

Title: Gain maximization leads to optimal exploration

Authors: *B. SI
Shenyang Inst. of Automation, Shenyang, China

Abstract: The conflicting goals of efficient reward estimation by exploration and exploitation of the actions determined to be the best require both, to explore efficiently and to trade-off exploration versus exploitation. Here we introduce a new performance measure tailored to finding the best action from which we derive an exploration policy maximizing the gain in the performance of estimation. We show that gain maximization naturally leads to optimal exploration in terms of the performance measure. Two heuristic exploration policies in reinforcement learning, namely error-based and counter-based exploration, can be obtained as policies maximizing gains of different cases of a generalization of this measure. Simulations of multi-armed bandits reveal, however, that they lead to suboptimal performance in selecting the best action. An upper bound on the sample complexity of the general gain-maximization selection strategy is established which provides stopping conditions for all gain-based exploration policies. Because these results rely on prior information realistically unavailable, a realistic gain-maximization selection policy is proposed which approaches the optimal exploration policy asymptotically. Using this exploration policy in a linear trade-off with

exploitation is shown to provide a new efficient solution to the reward-maximization problem, by fast switching to exploitation after sufficient exploration.

Disclosures: B. Si: None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.06/SS46

Topic: F.01. Human Cognition and Behavior

Title: Microsaccades: An involuntary tell of evolving economic decisions

Authors: *M. C. DORRIS[1], G. YU[1], B. XU[1], Y. ZHAO[1], B. ZHANG[1], M. YANG[1], J. Y. Y. KAN[1,2], D. M. MILSTEIN[2], D. THEVARAJAH[2]
[1]Inst. of Neuroscience, Shanghai Inst. For Biol. Sciences, CAS, Shanghai, China; [2]Dept. of Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: Microsaccades are small amplitude (typically <1°), unconscious and largely involuntary ballistic eye movements that occur when attempting to fixate gaze. Although initially thought to be random, recently it has been established that they are influenced by sensory stimuli, attentional processes and certain cognitive states. Whether decision processes influence microsaccades is unknown. Here we use two economic tasks to see whether microsaccades predict evolving saccade decisions. Volitional saccade choices of monkey and human subjects provided a measure of the subjective value of targets. Importantly, microsaccades were analyzed during a period of complete darkness, so decision processes, rather than sensory or attentional cues, could influence them. As saccadic choice approached, microsaccade direction became: 1) biased towards targets as a function of their subjective value, and 2) predictive of upcoming voluntary choice. Our results indicate that microsaccade direction is influenced by, and is a reliable tell of, evolving saccade decisions. Our results are consistent with dynamic decision processes within the midbrain superior colliculus; that is, microsaccade direction is influenced by the transition of activity towards caudal saccade regions associated with high value and/or future choice. Normally there should be strong selective pressures against such reliable tells lest they be exploited by competitive rivals. We propose that their small size has made microsaccades undetectable and thus immune to these evolutionary forces. This undetectability, however, is changing with the advent of accurate, inexpensive and mobile eye-tracking technology that is becoming increasingly commonplace in everyday electronic devices.

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Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.07/SS47

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP-102662

CFI

FRSQ

GRSNC

Title: Are value-based action choices made by a central executive or through a distributed consensus?

Authors: A. NAKAHASHI, *P. E. CISEK
Univ. of Montreal, Montreal, QC, Canada

Abstract: Classical theories suggest that all decisions are made within a cognitive central executive (CE) that is separate from sensorimotor control. Consistent with this, several studies have shown that neural activity in orbitofrontal (OFC) and ventromedial prefrontal cortex (vmPFC) represents outcome values in an abstract "common currency" that reflects subjective preferences. However, value-related activity has also been observed in many regions involved in sensorimotor control. This has led to the hypothesis that, at least when deciding between concrete actions, the brain represents potential actions in parallel and they compete against each other within the sensorimotor system. In this model, information about outcome values is one among several sources of bias into this competition and the decision emerges as a "distributed consensus" (DC) across a large fronto-parietal circuit. Testing the CE and DC models is challenging because it is notoriously difficult to dissociate neural activity pertinent to a choice from activity that causally determines the choice. Here, we attempt to meet this challenge using a value-based decision task in which two different kinds of information must be combined to estimate the reward value of a given action choice: "bottom-up" cues based on luminance; and "top-down" cues based on a learned mapping of stimulus orientation. In a subset of trials, these

two sources of information are in conflict (e.g. bottom-up cues favor one target while top-down cues favor the other), so that there is no better choice but a choice must still be made. The CE model predicts that regardless of which choice is made in conflict trials, it will be driven by activity from prefrontal regions and be reflected in sensorimotor areas with a consistent sequence of activation. In contrast, the DC model predicts that the sequence will be choice-dependent: parietal-to-frontal when subjects resolve the conflict to favor bottom-up information, and frontal-to-parietal when they resolve it to favor the top-down cue. The DC model further predicts that reaction times will be shorter when subjects favor bottom-up information than when they favor top-down cues. In this poster, we will present behavioral data from human subjects and one rhesus monkey. In almost all cases reaction times during conflict trials are significantly shorter when the decision is driven by the bottom-up than by the top-down information. This is consistent with the DC model, but a more conclusive test of the models will require analyses of neural data.

Disclosures: A. Nakahashi: None. **P.E. Cisek:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.08/SS48

Topic: F.01. Human Cognition and Behavior

Title: Dynamic adaptation of subjective values within a choice set

Authors: *P. W. GLIMCHER, M. KHAW
Ctr. Neural Sci., New York Univ., NEW YORK, NY

Abstract: Modern theoretical frameworks on decision-making highlight the stochastic nature of the decision variables guiding choice: subjective values/utility (Webb et al, 2013; McFadden, 1980). Recent results suggest that subjective value modulations in neurons, as with sensory representations, are encoded in a relative and temporally dynamic manner (Louie et al., 2011; LoFaro et al. in sub.); thus, other potential temporal dynamics of value and behavioral effects (e.g., Louie et al., 2013) warrant investigation. Here, we ask whether it is possible to systematically bias preferences with a value-based adaptation manipulation that mimics the effect of sensory after effects. We hypothesize that the subjective values of goods can be increased or decreased after prolonged exposure of the subject to specific choice sets. We test these predictions with a behavioral choice experiment employing two adaptation blocks. Human

subjects make choices between all pairs of 30 consumer goods. Using the rank ordering of their revealed preferences, we separate the array of goods for each subject into high, low, and medium-valued items. Subjects are then presented with high and low adaptation blocks in which they were asked to simply rate the appeal of each good individually. High adaptation blocks contain the top 10 ranked (i.e., high-value) goods along with 5 medium-value goods. Similarly, low adaptation blocks are comprised of the last 10 ranked items along with 5 medium-value goods. Medium-value goods were chosen to be every other good in its range, so as to preserve the average rank included within each adaptation phase. Each adaptation block includes 15 presentations of each good, randomly interleaved within their respective sets. Immediately after completion of both adaptation blocks, subjects once again performed all pairwise choices of the complete choice set. Subjects show a systematic drift in their ratings of the desirability of medium-valued goods during the adaptation blocks, as predicted by our neurobiological theory of value representation. That is, medium-value goods presented with high-value items come to be rated as be less attractive by the end of the block, and vice versa for items placed in low-value blocks. The final choice experiment revealed increased rank for medium-value items adapted with low-value items, along with a reduction in rank for medium items that were previously grouped with high-ranked items. In sum, we describe a novel context-dependent choice effect based on the dynamics of subjective value signals. The study presents novel insights into value adaptation and provides for neurally-inspired applied strategies in consumer decision-making.

Disclosures: P.W. Glimcher: None. **M. Khaw:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

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Program#/Poster#: 459.09/SS49

Topic: F.01. Human Cognition and Behavior

Support: James S. McDonnell Foundation Grant

Title: VMPFC tracks subjective value according to choice context during decisions about abstract reinforcers

Authors: *C. FINNERTY, S. J. HANSON, C. HANSON
Rutgers Univ., Newark, NJ

Abstract: Decision making studies have investigated the goal value of rewards in task contexts such as choosing monetary gambles in order to maximize payout. No studies have examined how reward value is calculated in contexts where reinforcers are abstract, hypothetical, and not related to tangible outcomes. The present study examines how value of abstract reinforcers is coded in the brain, using a novel task in which hypothetical choices for exemplars like preferred hobbies or travel are individualized to be perceived as higher or lower in value. Choice framing was manipulated by asking "which do you like more/less" while 15 subjects (8 female, mean age =25.47, SD=4.37 years) chose between examples in their preferred category while undergoing fMRI scanning. Participants reported the average number of hours per week (HPW) they actually engaged in preferred activities, as well as their desired HPW if time or money were no object. Number of desired (but not actual) HPW correlated with reaction time in both positive ($t = -3.2576$, $df = 13$, p-value = 0.006) and negative framing ($t = -3.5328$, $df = 13$, p-value = 0.004). Desired HPW was used a predictor of brain activation, and related activity was observed in the insula, putamen, and amygdala in positive framing, and ventromedial prefrontal cortex (VMPFC), anterior cingulate (ACC), and superior frontal gyrus (SFG) in negative framing. This network of regions was analyzed using a search based Bayes connectivity method, IMaGES. This method returns a graph of the connections between regions as well as the direction of temporal influence. Connections common to both conditions included VMPFC-ACC, VMPFC-putamen, amygdala-putamen, putamen-insula, and SFG-insula. Connections that changed directions between conditions included VMPFC-SFG and ACC-insula. There was a unique connection between amygdala-SFG in positive framing. These results demonstrate that value sensitive areas of the brain respond to changes in perceived value of abstract reinforcers, and this activation is also affected by task context in terms of both magnitude differences and changes in network connectivity. Network dynamics in value sensitive regions (ACC, VMPFC) showed the greatest connectivity changes between conditions. Desired HPW measured as a predictor of brain activation resulted in more limbic activation in positive framing and more cortical regions for negative. Decision value computations for abstract reinforcers may thus be calculated differently depending on context - whether framing is negative (rejecting a less preferred option) or positive (selecting a preferred option) - as well as the perceived value of stimuli.

Disclosures: **C. Finnerty:** None. **S.J. Hanson:** None. **C. Hanson:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.10/SS50

Topic: F.01. Human Cognition and Behavior

Support: R01 HL102119

R21 DA032022

P30 NS045839

Title: Effects of sleep deprivation on brain function at rest and during a gambling task

Authors: *N. MA[1], Z. FANG[1], S. ZHU[1], S. HU[1], J. DETRE[1], D. DINGES[2], H. RAO[1]
[1]Neurol., [2]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Sleep loss impairs essential cognitive performance including attention, working memory and decision making. Numerous studies using BOLD fMRI have revealed significant disruption of brain activation during various cognitive tasks after sleep deprivation (SD). However, BOLD contrast lacks absolute quantification of neural activity. Accordingly, it is impossible to determine whether changes in brain activation following SD are due to changes in baseline neural activity, or during specific tasks, or both. In the current study, we used arterial spin labeling (ASL) perfusion fMRI to quantify cerebral blood flow (CBF) after normal sleep and SD and dissociate the effects of SD on resting and working brain function during a gambling task. Methods: Twenty-nine healthy subjects (14 female, age 21-50 yrs) participated in a 5-day and 4-night SD study in which they were scanned three times at rest and during performing a Iowa Gambling Task (IGT) task: a first baseline (BS) scan after 9h normal sleep, a SD scan after 24h without sleep, and a third scan after two consecutive nights (20h) of recovery sleep (RS). Data were analyzed by SPM8 and the Grocer toolbox. Results: Compared to BS and RS, SD significantly reduced resting CBF in multiple brain regions, including bilateral thalamus, fronto-parietal attention network, and the default mode network (DMN), but increased regional CBF in bilateral occipital lobe, auditory cortex and sensorimotor cortex. During the IGT, a similar pattern of CBF changes was observed after SD compared to BS and RS, with smaller CBF increases in bilateral occipital lobe and smaller CBF decreases in the thalamus, attention network, and DMN. The interactions showed significantly reduced CBF activation in the occipital cortex and increased CBF activation in the thalamus and DMN. Conclusion: Both increased and decreased regional CBF were observed after SD compare to rested wakefulness, demonstrating heterogeneous effects of sleep loss on regional brain function. However, the pattern of absolute CBF changes is opposite to that of relative activation changes in the thalamus, DMN, and occipital cortex after SD, indicating the importance of using absolute and quantitative CBF measurements to dissociate the effects of sleep loss on resting and working brain function.

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Poster

459. Human Decision-Making: Value

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Topic: F.01. Human Cognition and Behavior

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Gift from Forrest and Sally Hoglund

CTSA Award UL1TR000001

Title: Eating alters insula functional connectivity to prefrontal and subcortical regions underlying behavioral and appetitive responses to foods

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Abstract: Previous research has identified a region of the dorsal mid-insula that is both selective for interoception of visceral sensations and responsive to the taste and sight of food stimuli. As this region is also near the first cortical enervation of vagal afferents carrying information about gastric volume after eating, we reasoned that the dorsal mid-insula might play a key role in providing postprandial signals of fullness to other brain regions that underlie cognitive control and appetitive responses to foods. To test this hypothesis we examined the resting-state fMRI scans of 55 obese adults taking part in a larger weight loss study. Subjects were scanned both before and after eating a standardized 500 kcal meal. We functionally defined the dorsal mid-insula seed region using data from prior published studies with a different cohort of subjects. The seed region was composed of the union of dorsal mid-insula voxels shown to be selective both for interoceptive attention to the viscera (Simmons et al., 2013, Human Brain Mapping), as well as responsive to gustatory stimulation and food pictures (Simmons et al., 2012, Nature Neuroscience). The individual subjects' maps showing correlation coefficients with the dorsal mid-insula seed region in both the pre and postmeal scans were converted to z-scores, and the differences between premeal and postmeal connectivity maps was calculated using a standard paired-sample t-test. Additionally, correlations were calculated between the change in postmeal - premeal z-scores and the change in self-reported hunger between meals. Resting-state functional

connectivity increased postprandially between the dorsal mid-insula and multiple regions in dorsal-lateral prefrontal cortex (BA 44 and 46), as well as in dorsal medial prefrontal cortex (BA 6). Additionally, we observed a region of the ventral striatum, including the accumbens areas, where changes in functional connectivity were related to changes in the subjects' subjective ratings of hunger. Specifically, those subjects who exhibited the largest reduction in hunger postprandially also exhibited the greatest increase in functional connectivity to the interoceptive insula. Taken together, these findings provide evidence for how postprandial interoceptive signals of fullness may influence activity in striatal and prefrontal regions that support post-meal changes in food motivation and feeding behavior.

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Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.12/SS52

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 AG033406

Title: The neural correlates of decision-making under uncertainty in monetary gains and losses

Authors: *L. RUDERMAN[1], M. A. GRUBB[2], A. TYMULA[3], D. B. EHRLICH[2], P. W. GLIMCHER[2], I. LEVY[1]
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Abstract: The choices we make reflect the *subjective values* we place on the options available to us. These subjective values are affected, among other things, by weighting of two separate factors: *risk attitude*, which reflects a trade-off between the probabilities of gains or losses and the magnitude of those gains and losses, and *ambiguity attitude*, which reflects the subject's sensitivity to uncertainty about these probabilities. We have recently documented the effect of risk and ambiguity attitudes in the domain of monetary gains on activation patterns in value-related areas, including the MPFC and the striatum. Gains and losses, however, are inherently different, which translates into different attitudes towards uncertainty; while individuals are risk-averse on average in the gain domain, they are risk-seeking in the loss domain. Additionally, individuals are ambiguity-averse in the gain domain, but ambiguity-neutral in the loss domain. Yet, only few imaging studies have addressed decisions between potential losses, and as far as

we know no study has examined ambiguous decision-making under losses. This study aims to explore the neural mechanism underlying decision-making under uncertainty in the loss domain, and compare it to the neural mechanism underlying the same decisions in the gain domain. We studied 31 subjects (age 19-35; 13 males) to assess their attitudes towards risk and ambiguity for monetary gains and losses. Subjects were asked to make choices between a guaranteed win (or loss) of a fixed monetary amount ($5) or playing a lottery that varied in probability (0.25, 0.5 and 0.75) and amount of money that could be won or lost (between $5 and $125). In some lotteries the odds of winning or losing were precisely specified (risky lotteries). In others, subjects only had partial information about these probabilities (25% to 75%), rendering them partially ambiguous. Subjects completed 120 choices on a computer and functional MRI was used to track neural activation while subjects completed additional 120 choices, for a total of 240 decisions. One choice was randomly picked for payment, so that subjects had to treat each trial as if they would be paid according to their choice. Our results reveal widespread effects of risk and ambiguity on activation patterns in value and choice related areas. In the gain domain the results replicate previous studies. In the loss domain we observed effects of risk and ambiguity on the activation pattern both in areas overlapping the ones implicated in gain processing and in unique areas. These results suggest the existence of a unified valuation system for gains and losses, as well as circuits which are specialized for one type of outcomes.

Disclosures: L. Ruderman: None. **M.A. Grubb:** None. **A. Tymula:** None. **D.B. Ehrlich:** None. **P.W. Glimcher:** None. **I. Levy:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.13/SS53

Topic: F.01. Human Cognition and Behavior

Title: A TMS study of dorsomedial frontal cortex role in transforming discounted value to actions in intertemporal choice

Authors: *C. A. RODRIGUEZ, B. M. TURNER, S. M. MCCLURE
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Abstract: Recent computational modeling, EEG and fMRI findings suggest that the dorsomedial frontal cortex (dmFC) implements a value integration process that translates delay discounted value into intertemporal choice behavior. However, it is still unclear if the dmFC truly plays a

causal role in this value-to-action transformation. We investigated the causal involvement of dMFC in value integration during intertemporal choice using transcranial magnetic stimulation (TMS). Participants made intertemporal choices after receiving real or sham repeated TMS pulses to the dmFC. We summarized the resulting differences in behavior using a Linear Ballistic Accumulation model of value integration in intertemporal choice. Our results demonstrate that the dmFC plays a causal role in translating delay-discounted value into intertemporal choice behavior that maximizes long-term subjective benefit through value integration.

Disclosures: C.A. Rodriguez: None. **B.M. Turner:** None. **S.M. McClure:** None.

Poster

459. Human Decision-Making: Value

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 459.14/SS54

Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation: University of Chicago Wisdom Research grant

Title: Market experience attenuates the endowment effect through modulation of anterior insula

Authors: *L. TONG[1], K. ASAI[1], K. J. YE[1], S. ERTAC[3], J. A. LIST[1], H. C. NUSBAUM[2], A. HORTACSU[1]
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Abstract: Consumers ask a greater price for goods that they own than they are willing to pay for otherwise identical goods. Traders_people who have substantial market experience buying and selling_have been shown in behavioral research to have a reduced endowment effect. But little is known about the mechanisms underlying this expertise modulation. The present study identifies decreased loss aversion as a potential mechanism by which experienced traders overcome the endowment effect. Experienced (n=18) and inexperienced (n=17) traders (experience in eBay, stock trading, or other professional selling activities) used a slider bar to report the maximum they would be willing to pay, and the minimum they would be willing to accept in exchange for different products. Then, they made a series of decisions to buy or sell those products at high- and low-prices while undergoing fMRI. A series of regressions revealed that (1) inexperienced participants exhibited on average 42% larger behavioral endowment effects in the slider

decisions than experienced participants, controlling for demographic and task-related effects, (2) the right anterior insula region of interest was more active in inexperienced than in experienced participants in response to offers during selling, but there was no significant difference during buying, and (3) insula percent signal change, particularly in response to low-ball offers during selling, positively predicts the behavioral endowment effect (all ps<0.05). A mediation analysis demonstrated that including insular activation in the regression reduced the direct effect of trading experience on the endowment effect to 28%, which was no longer significant (p=0.13). Trading experience modulated insula activation when selling, but not buying consumer goods. This suggests that frequent trading may reduce an individual's susceptibility to the endowment effect through decreasing negative affect associated with selling away products that they own, particularly when faced with low-ball offers.

Disclosures: L. Tong: None. **K. Asai:** None. **K.J. Ye:** None. **S. Ertac:** None. **J.A. List:** None. **H.C. Nusbaum:** None. **A. Hortacsu:** None.

Poster

459. Human Decision-Making: Value

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Topic: F.01. Human Cognition and Behavior

Support: NRF MEST Korea 20100018840

NRF MEST Korea 2011-0031867

Title: Decoding of like/dislike intentions using single-trial electroencephalograms

Authors: *J. CHOI, K. CHA, K. KIM
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Abstract: The purpose of this study is to investigate whether intentions can be identified, i.e., 'decoded' from non-invasive electroencephalogram (EEG). In particular, we tried to decode from single-trial EEG waveforms whether a subject's response to affective visual stimuli was 'like' or 'dislike'. 17 healthy university students participated in the experiment. The pictures consisted of pleasant, unpleasant and neutral pictures selected from the International Affective Pictures System (IAPS). The subjects were requested to decide whether they 'liked' or 'disliked' each picture, then maintain the intention in mind, and finally respond with button press.

Multichannel EEGs were recorded during the task. EEGs during the 'intention in mind' period (before the button press) were segmented. Normalized event-related spectral perturbation (ERSP) maps were constructed, and the spectral, temporal and spatial characteristics showing significant power differences between 'like' and 'dislike' were found by statistical comparison. The powers within such time-frequency ranges at selected electrodes were determined to construct feature vectors for pattern classification. Quadratic support vector machine was adopted as a pattern classifier along with sequential feature selection for dimensionality reduction. The alpha power was significantly different between 'like' and 'dislike' (group analysis). The differences were observed mainly at frontal regions in 2500-4000 ms period, corresponding to the interval of holding the choice in memory. The alpha power was stronger for 'like' than 'dislike'. By cross-validation, the trained pattern classifier showed classification accuracy as high as 62.8 %. Our findings indicate that the intentions regarding binary decision of like/dislike in response to the affective pictures can be decoded from single-trial scalp EEG.

Disclosures: J. Choi: None. **K. Cha:** None. **K. Kim:** None.

Poster

460. Human Social Cognition I

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Support: Associates of the Child Study Center

Allied World

Autism Science Foundation

Hilibrand Foundation

NIMH

Autism Speaks

Harris Family Professorship

Title: Pivotal response treatment alters brain function in children with autism

Authors: *G. ROSENBLAU, H. FRIEDMAN, B. VANDER WYK, K. PELPHREY, P. VENTOLA
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Abstract: Autism Spectrum Disorders (ASD) are characterized by severe social deficits (Levy, Mandell, & Schultz, 2009). There are several social skills interventions for individuals with ASD (Wang & Spillane, 2009), but whether and how they affect the underlying neural mechanisms remains largely unknown. Pivotal response treatment (PRT) is an empirically validated behavioral treatment promoting social skills in individuals with ASD (Voos et al., 2013). We aim at identifying the neural and eye-tracking mechanisms supporting social communication improvements in children receiving PRT vs. a wait-list control condition. We are randomly assigning 4- to 6-year-old children with ASD (*N*=20) to either a waitlist control group (WTC, *n*=10) or the PRT group (*n*=10). Additionally, we include typically developing children (TD, *n*=20, matched for age, sex and IQ) as a point of reference for the interpretation of changes in brain function and visual scan paths. All children are assessed twice, at time 1 (T1) and time 2 (T2) separated by a four-month interval, during which the PRT group receives treatment. Preliminary eye-tracking data from 8 children with ASD (6 males) pre and post PRT indicates that at T2, the proportion of fixations on the mouth region significantly increases, relative to T1. FMRI analysis including 10 children with ASD (9 male) at T1 yields activity of the right fusiform gyrus, and the bilateral lateral occipital cortex for faces vs. houses. Analysis of treatment effects (T2 > T1) reveals significant increases in activity in the medial prefrontal cortex (MPFC) for social stimuli (faces vs. houses) in PRT (*n*=5) vs. WTC (*n*=5) group. Analyses of PRT-related effects indicate that the treatment modulates behavior and brain function. In particular, the MPFC has been closely linked to consciously engaging in social cognition (e.g., taking another's perspective into account (Amodio & Frith, 2006)). Increased activity in the MPFC after treatment might underlie the positive effects of PRT on social behavior.

Disclosures: G. Rosenblau: None. **H. Friedman:** None. **B. Vander Wyk:** None. **K. Pelphrey:** None. **P. Ventola:** None.

Poster

460. Human Social Cognition I

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Program#/Poster#: 460.02/SS57

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26119529

KAKENHI 25700015

Title: Event-related potential (ERP) for gaze perception and its relation to social anxiety tendencies

Authors: *Y. TSUJI, S. SHIMADA
Meiji Univ., Kawasaki Kanagawa, Japan

Abstract: We used event-related potential (ERP) to examine the effects of social anxiety tendencies on eye gaze perception. Avoidance or excessive fear is a defining feature of social anxiety disorder (SAD) or social phobia (SP) in a situation associated with being evaluated or embarrassed by others. Especially, gaze of others is known to frequently induce social anxiety. Sixteen healthy adult subjects (2 females, aged 20.7 ± 0.68, mean ± SD) participated in this study. Participant's level of social anxiety was examined by means of the Japanese version of the Liebowitz Social Anxiety Scale (LSAS-J). The experimental stimulus was either a picture of direct or averted eye gaze, or a scrambled control image. In each trial, the experimental stimulus was displayed for 500ms, and then fixation cross appeared for 1500-2000ms (jittered). An experimental session consisted of three blocks of 20 trials, and the subject underwent three experimental sessions. The same conditional stimuli were used within each block. Electroencephalogram (EEG) signals were recorded from 26 scalp sites, located according to the extended international 10/20 reference system. The amplitude and latency of P200 at Fz were entered into one-way ANOVAs with the factor of conditions (gaze, averted vs. control). The ANOVA revealed a significant main effect of amplitude ($F(2,30) = 12.8$, $p<0.05$) and latency ($F(2,30) = 7.2$, $p<0.05$). Post hoc analysis (Tukey's honestly significant difference; HSD) revealed that there were significant differences between the direct gaze and control conditions ($p<0.05$) as well as the averted gaze and control conditions ($p<0.05$). The correlation between LSAS-J score and P200 latency was examined by Spearman rank correlation (rs). We found a negative correlation between LSAS-J and P200 latency only in the direct gaze condition (correlation coefficient $rs =-0.54$, $p<0.05$). There was no significant correlation between LSAS-J and P200 amplitude. This result suggests that other's direct gaze is forwardly processed in the subject who has high tendency towards social anxiety.

Disclosures: Y. Tsuji: None. **S. Shimada:** None.

Poster

460. Human Social Cognition I

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Support: The Wyncote Foundation

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NIH AG043503

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Title: Neural basis of social coordination deficits in patients with behavioral variant frontotemporal dementia (bvFTD)

Authors: *M. HEALEY, S. GOLOB, N. SPOTORNO, R. CLARK, C. MCMILLAN, M. GROSSMAN
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Abstract: Conversational partners establish shared mental representations to mediate common understanding. Game theory, rooted in principles of strategy and decision-making, refers to this as social coordination. Patients with bvFTD offer a unique window into the neural mechanisms of social coordination. Characterized by inappropriate social behavior and executive limitations, bvFTD is a rare neurodegenerative disease associated with progressive atrophy in frontal and temporal regions. To probe social coordination in bvFTD, we developed a novel task in which patients (N=12) and matched controls (N=14) were shown two-scene stories illustrating the movement of a target toy that was embedded in a shelf of competing objects sharing color, size, or pattern features. Participants had to describe the scene with sufficient detail so a conversational partner could correctly identify the moving toy. Trials varied in the amount of information available to the partner: in common ground trials, the partner had equal access to visual information; in colorblind trials, the partner was colorblind; and in privileged ground trials, there was a physical obstruction partially blocking the partner's view. The latter two conditions put increasing demand on perspective-taking ability. When conditions were compared, we saw that patients were impaired on colorblind trials, which required subjects to mentally represent their partner's knowledge, but not on privileged ground trials, in which there was a physical reminder of the partner's limited knowledge. High-resolution structural MRI related performance on colorblind trials (with common ground trials as a baseline) to

orbitofrontal (OFC) and medial frontal (MFC) atrophy, suggesting a crucial role of these areas in cognitive perspective-taking. Responses were also scored as precise, superfluous, or insufficient, depending on adjective use. This analysis showed that patients (47.97 ± 8.65%) gave more insufficient responses (e.g. when two pigs were present, "pig" instead of "red pig") relative to elderly controls (21.56 ± 11.05%, $p<0.001$). This was negatively correlated with forward digit span ($p<0.01$) and Trailmaking Test, Part B ($p<0.05$). The rate of insufficient responses was related to MFC and OFC atrophy, as in the colorblind analysis, as well as atrophy in dorsolateral prefrontal cortex (DLPFC). This suggests that DLPFC plays a role in resource demands associated with maintaining object features in an active cognitive state during processing. In sum, our results indicate that social coordination deficits in bvFTD are due to impaired perspective-taking and executive dysfunction, due to frontal atrophy.

Disclosures: **M. Healey:** None. **S. Golob:** None. **N. Spotorno:** None. **R. Clark:** None. **C. McMillan:** None. **M. Grossman:** None.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: DFG Grant EXC302

Title: Sharing self-related information on social media is associated with intrinsic functional connectivity of cortical midline brain regions

Authors: ***D. MESHI**[1], L. MAMEROW[1], E. KIRILINA[2], C. MORAWETZ[1], D. MARGULIES[3], H. R. HEEKEREN[1]
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Abstract: Human beings are social animals who share information about themselves with others. We do this, for example, by talking face-to-face, conversing on the phone, or using social networking websites. Regardless of the mode of communication, humans display inter-individual differences in the degree to which they share self-related information. Brain networks involved in self-related cognition have been identified, especially via the use of resting-state experiments,

but importantly, the neural circuitry underlying individual differences in the sharing of self-related information is currently unknown. Therefore, we set out to investigate the intrinsic functional organization of the brain with respect to participants' degree of self-related information sharing. To do this, we recruited 35 healthy, right-handed participants (14 male) between 19 and 34 years of age (mean=25.7, s.d.=3.7). We collected functional neuroimaging data (Siemens, 3T) for two resting-state runs of 7 minutes and 40 seconds each. After scanning, participants were given a questionnaire to assess their degree of self-related sharing on Facebook. The sharing of self-related information on social media was employed as a proxy for the sharing of self-related information in the real world. We conducted seed-based correlation analyses in cortical midline regions previously shown to be involved in self-referential cognition: the medial prefrontal cortex (MPFC), central precuneus (CP), and caudal anterior cingulate cortex (CACC). We examined if functional connectivity between these regions and the rest of the brain was associated with participants' degree of self-related information sharing. Analyses revealed associations between the MPFC and right dorsolateral prefrontal cortex (DLPFC), as well as the CP with the right DLPFC, the left lateral orbitofrontal cortex and left anterior temporal pole. No associations with self-related information sharing were observed with the CACC. All results were whole-brain FWE cluster-level corrected at $p < 0.05$ after setting the voxel-level uncorrected threshold to $p < 0.001$. Our findings extend our present knowledge of functional brain connectivity, specifically demonstrating how the brain's intrinsic functional organization relates to individual differences in the sharing of self-related information. This research is an important step towards understanding the human social behavior of disclosing information about the self.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: DE130100120

DP130100559

Title: The neuroscience of inspirational leadership: Why leaders should care about shared group membership

Authors: *P. MOLENBERGHS[1], G. PROCHILO[1], N. K. STEFFENS[1], H. ZACHER[2], S. A. HASLAM[1]
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Abstract: Effective leaders are believed to inspire followers by providing inclusive visions of the future that followers can identify with. In the present study, we examined the neural mechanisms underlying this process, testing key hypotheses derived from transformational and social identity approaches to leadership. While undergoing fMRI, we presented supporters (N= 40) from the two major Australian political parties (Liberal vs. Labor) with inspirational collective-oriented and non-inspirational personal-oriented statements made by ingroup and outgroup leaders. Imaging data revealed that inspirational (rather than non-inspirational) statements from ingroup leaders were associated with increased activation in the bilateral rostral inferior parietal lobule, pars opercularis, and posterior midcingulate cortex: brain areas that are typically implicated in semantic information processing. In contrast, for outgroup leaders greater activation in these areas was associated with non-inspirational statements. In addition, non-inspirational statements by ingroup (but not outgroup) leaders also resulted in increased activation in the medial prefrontal cortex, an area typically associated with reasoning about a person's mental state. These results show that followers processed identical leadership statements in line with their pre-existing beliefs (i.e., more inspirational statements by ingroup leaders and more non-inspirational statements by outgroup leaders), thus emphasizing the importance of a shared identity in inspirational leadership communication.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: UNIMIB 2011FAR

Title: How social bias (prejudice) affects memory for faces: An electrical neuroimaging study

Authors: *A. M. PROVERBIO[1], F. LA MASTRA[2], R. ADORNI[2], A. ZANI[3]
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Abstract: When socially interacting with people we normally make inferences about their personal traits on the basis of their appearance. From these inferences a potential prejudice may arise that can positively or negatively bias our interactions with them. Not much is known about the effects of prejudices on face perception and the ability to recognize people faces. The ability to code and remember human faces was investigated by recording brain Event-Related Potentials (ERPs) from 128 scalp sites in 16 healthy volunteers during a face coding and a memory task. In the first session (encoding) volunteers viewed 200 human faces associated with a short fictional story that described anecdotal positive or negative traits about each person (see an example of a negatively biased face in Fig. 1). In the second session (memory retrieval), they underwent an old/new memory recognition test, in which they had to distinguish 100 new faces from the previously shown ones. ERP data relative to the encoding phase showed an anterior N400 larger to negatively (vs. positively) biased faces, probably indexing a deeper processing of faces linked to unpleasant social traits. In the memory task ERP recorded to new faces elicited a larger FN400 as compared to familiar faces. In addition, old faces (and especially positive ones) elicited a larger Old-New parietal response than new faces, in the form of an enlarged LPC component. The analysis of the inverse solution swLORETA applied to LPC response (450-550 ms) indicated that recognition of old faces (as compared to new faces) was associated with a stronger activation of FFA and OFA areas, Superior Frontal Gyrus and Middle Temporal Gyrus. The degree of these activations, expressed in magnitude, was higher for negatively connoted faces. Furthermore, it was observed that memory recognition of negatively connoted faces strongly activated the limbic and parahippocampal areas and the Posterior Cingulate cortex.

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Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

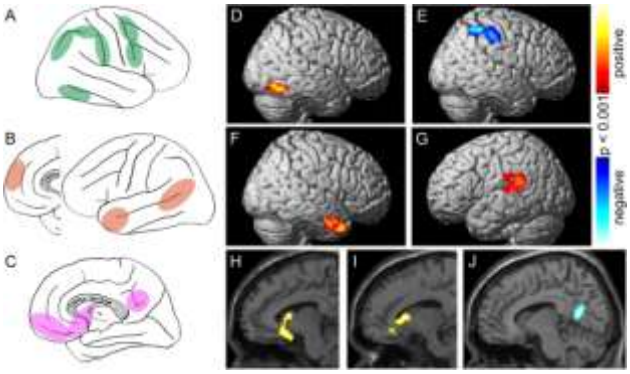
Support: JSPS KAKENHI 26118702

Title: Personality and activation during self-face recognition: Three aspects

Authors: ***M. SUGIURA**[1,2], Y. KOTOZAKI[1], A. SEKIGUCHI[1,3], C. M. MIYAUCHI[1,4], S. HANAWA[1], S. NAKAGAWA[1], T. ARAKI[1], R. KAWASHIMA[1]

[1]IDAC, Tohoku Univ., Sendai, Japan; [2]IRIDeS, Tohoku Univ., Sendai, Japan; [3]ToMMo, Tohoku Univ., Sendai, Japan; [4]The Univ. of Tokyo, Tokyo, Japan

Abstract: Brain activation during self-face recognition has at least three aspects. Activation in the visual, somatosensory, and motor association cortices (Fig. A) may reflect cognitive load to resolve gap or conflict between perceived self-face and its mental representation. Deactivation of the areas related to the processing of others' mental states (Fig. B) may reflect sparing of a social attentional process that is necessary for any faces except for the self-face. Activation observed depended on social context in the cortical midline structure (CMS) and reward system (Fig. C) may reflect context-dependent social value of the self. We were interested in whether these three aspects also make sense on the individual difference in activation. The protocol was approved by a local ethical committee. FMRI data during the recognition of self-face and an unfamiliar face as well as 50 scores of self-cognition-related or general personality traits were collected. A significant correlation was explored using voxel-wise regression analysis using the contrasts of activation for two faces from 52 participants ($p < .05$, corrected). A positive correlation with the score on the traits reflecting inefficient conflict processing (Premature Self-Defense [Fig. D] and low Openness [Fig. E]) was identified in the visual association cortices. That with the score on the tendency to pay social attention to one's own behavior (Fantasy in Interpersonal Reactivity Index [Fig. F] and Beta-type Pride in Test of Self-Conscious Affect [Fig. G]) was identified in the areas related to the processing of others mental states. That with the score on the tendency to care for the context-dependent social value of the self (Public Self-Consciousness [Fig. H], Extraversion [Fig. I], and low Self-Monitoring [Fig. J]) was identified in the CMS or reward system. The finding is thus supportive of the three-aspect view of the individual difference in activation during self-face recognition.



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Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

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JSPS Grant #26730075

Title: Neural correlates of intention and relationship attribution of animated motion: An fMRI study

Authors: *K. YAOI[1], T. MINAMOTO[2], M. OSAKA[2], N. OSAKA[1]
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Abstract: In a social context, we can interpret others' intention and emotion by their bodily motion or expression. This ability plays a very important role for our sociality. On the other hand, animated movements of simple geometric shapes also can readily be interpreted as depicting social events in which animate agents are engaged in intentional activity (Osaka et al., 2012). Recently, some studies have investigated cognitive and neural basis of interpreting intention of simple moving object like rectangle or triangle. However, little research has been conducted on neural correlates of interpreting "relationship" between objects from their movements. By investigating about intention and relationship attribution of animated motion, it would be capable of uncovering our basic functions to understand others' mind in real social context. In this study, therefore, we manipulated shape animations while measuring associated brain activity using fMRI. We created animation movies in a manner similar to the Heider & Simmel (1944) pattern and prepared four conditions. First, in the "clash" condition, two triangles (red, blue or yellow) clashed hard each other, one interrupted the other and so on. In the "chummy" condition, on the other hand, two triangles moved like picking playfully or cooperating each other. Furthermore, in the "mix" condition, we picked up and mixed one triangle from "clash condition" and another from "chummy condition" animation. Therefore, each triangle looked like moving with intention, but did not relate each other. Fourth, in the "low-intention" condition, two triangles showed low-intentional movement, for example, going straight at the constant speed or keeping on turning on site. In MRI scanner, 17 participants were required to rate intention and with intimacy on a 7-point scale. fMRI result indicated that the "clash" condition showed greater activation in the right superior temporal sulcus, whereas the "chummy" condition evoked greater activation in the left supramarginal cortex than the "low-intention" condition. Then, from the direct comparison between the "clash" and the "chummy"

conditions, the “clash” condition evoked greater activation in the right temporo-parietal junction, whereas the “chummy” condition showed greater activation in the cerebellum, the left inferior parietal lobe and some other regions. These results may indicate that there are some cognitive or neural dissociation between interpreting intention and relationship from simple moving objects.

Disclosures: **K. Yaoi:** None. **T. Minamoto:** None. **M. Osaka:** None. **N. Osaka:** None.

Poster

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Title: Perception through action: Role of the left sensorimotor cortex in facial categorization

Authors: *A. PERRY[1,2], J. DEVRIES[2], H. KIRSCH[3], E. CHANG[4], N. CRONE[5], R. T. KNIGHT[1,2], A. Y. SHESTYUK[1,2]
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Abstract: Facial expressions hold important information about the other's intentions, motivations and feelings. Embodied theories of cognition posit that automatic sensorimotor transformations (i.e., simulation) are required to understand others’ facial expressions through engagement of sensorimotor (SM) cortical areas. There is evidence of involvement of SM cortices in recognition of facial expressions. However, most of these studies are limited to the right hemisphere and do not provide evidence that facial categorization (i.e., evaluation of a

static representation of a facial movement) recruits the same brain regions involved in the motor execution of facial movements. The exact timing of SM recruitment during face categorization also remains unexplored. To address these questions, we utilized the spatial and temporal advantages of intracranial cortical recordings (electrocorticography) to test whether and when neuronal populations in SM regions are activated during facial categorization tasks as well as during execution of facial movements. We tested 4 participants with intractable epilepsy implanted with electrodes over the left SM cortex undergoing pre-operative monitoring. Patients performed emotion categorization (angry vs. neutral and happy vs. sad) and gender categorization tasks, as well as a motor execution task (either making facial expressions or generating verbal responses to a cue). Increases in spectral power in the high gamma range (70-150 Hz; $p<.01$ above baseline for at least 100ms) were used to index cortical activation. Our results show SM cortex activation (middle to inferior pre-central and post-central gyri) during both emotion and gender categorization. Importantly, 38% (16/42) of the sites that were active for categorization of facial stimuli were also active during the motor execution task; 53% (16/30) of the sites that were active during motor execution were also active for face categorization, suggesting that face categorization engages brain areas directly involved in facial motor execution. SM cortex activation was evident at various temporal stages – as early as 75ms post-stimulus or as late as 50ms before response and was not specific to either emotion or to the type of categorization (emotion or gender), suggesting a broad engagement of SM cortex during general face processing. These results extend the simulation theory by identifying the left SM cortex as a key region not only during facial movements but also during non-motor evaluation of static face stimuli.

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Poster

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Support: Rothschild Fellowship

National Postdoctoral Award Program for Advancing Women in Science

DARPA-BAA-12-03-SBIR Phase II “Narrative Networks”

Title: How does the brain represent different ways of understanding the same story?

Authors: *Y. YESHURUN[1], S. SWANSON[2], J. CHEN[1], E. SIMONY[1], C. HONEY[5], C. LAZARIDI[3], U. HASSON[4]
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Abstract: Understanding a narrative requires the creation of mental representations of the situations in the story. Such situation models are composed of prior knowledge (context) as well as ongoing analysis of the intentions, perceptions, emotions, and beliefs of the characters. In this functional magnetic resonance imaging study we set out to test how different contexts modify the neural representation of the same story. Forty subjects were presented with an auditory story designed to contain ambiguous information about character relationships and mental states. To disambiguate the story we provided subjects with two different brief introductions (contexts) pointing toward one of the two interpretations. Half of subjects received one introduction and half received the other introduction before listening to the story. We tested how differentiable neural responses were for the two story interpretations using Linear Support Vector Machine classifier. We classified the data across the whole brain, as well as in specific ROIs defined by a separate Theory of Mind localizer. Behavioral results suggested comparable levels of story comprehension between the two groups ($p=0.8$), together with a significant difference ($p<10\text{-}9$) in reported interpretation of the narrative. Brain data revealed subset of regions, including precuneus, right TPJ and bilateral hippocampus, that distinguished between the two interpretations. More so, some periods of the story were more informative in separating the two contexts, and the informative periods were not the same for all regions. These results identify regions that their activity over time carried information about high-level aspects of the situation, and show that changes in these constructs can be detected even when sensory input is held constant.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: IT R&D program of MISP/KEIT KI10045461

Title: Coherent groupwise responses of brain rhythms in EEG during watching a movie

Authors: *D. KANG[1], J. KIM[2], Y. SHIN[3], D.-P. JANG[3], S.-P. KIM[1]
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Abstract: Neurocinematics has recently emerged as an interdisciplinary field, aiming to understand social cognitive interactions with cultural environments and effects of cinema experiences on cognitive processes in human populations. During watching a movie composing of multi-modal stimuli, viewers may experience dynamical changes in their cognitive and emotional states. Such dynamical cognitive and affective states are likely to be best examined at the level of a population of people as the movie is supposed to elicit social and cultural responses in viewers. A number of studies have focused on finding coherent responses of a group of people to a movie using metrics such as the inter-subject correlation (ISC) in functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) data. However, little is known about coherent group-wise responses in specific brain rhythms during watching a movie. Also, it would be necessary to have concurrent measurements of brain activity of multiple people in order to investigate sociocultural effects of the movie. In this study, we simultaneously measured brain activities of multiple subjects while they were watching a Korean movie (The Chaser, 2008, Bidangil, Inc., South Korea) together at the same place. We analyzed spectral ISC in five distinct frequency bands: Delta (2~4Hz), Theta (4~8Hz), Alpha (8~13Hz), low Beta (13~18), and high Beta (18~23Hz). A non-parametric permutation analysis was used to assess statistical changes in ISCs over time. We found several time periods throughout the running time of the movie in which the ISCs of all frequency bands exhibited significant changes ($P<0.05$). We examined the contents within these periods and found that they included information essential to the development of the plot or the maintenance of emotional intensity. Our results demonstrate that the spectral inter-subject correlation analysis of EEG signals simultaneously recorded in multiple people may provide insights on how a movie elicits common brain responses in accordance with the contents of the movie.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Subtype- and phenotype-specific altered functional connectivity of social anxiety

Authors: *S. KAJIMURA[1], T. KOCHIYAMA[2], R. NAKAI[3], N. ABE[3], M. NOMURA[1]
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Abstract: Recent research has shown that social anxiety disorder (SAD), which shows excessive fear and anxiety of negative evaluation, is accompanied by abnormalities in brain function (Liu et al., 2013). However, few studies have focused on the differences among SAD subtypes, i.e., social interaction and performance anxiety (Stein & Deutsch, 2003). It is important to regard SAD as a multidimensional disease that requires personalized intervention since a relatively large number of patients do not benefit from either psychotherapy or medication (O'Toole & Pedersen, 2011). We investigated the common / specific resting functional connectivity (FC) which was related with SAD subtypes and the phenotype. Of the 20 participants who completed questionnaires and resting functional magnetic resonance imaging (fMRI), one subject was excluded because of excess head motion; thus, 19 participants (7 female, 20.9 ± 1.8 years) were included in our analysis. Fear of negative evaluation, social interaction, and performance anxiety were evaluated by the BFNES (Leary, 1983), SIAS (Mattick & Clarke, 1998), and SPS (Mattick & Clarke, 1998), respectively. Preprocessed fMRI data was parcellated into 90 ROIs based on Automated Anatomical Labeling (AAL; Tzourio-Mazoyer et al., 2002), and a time series was obtained by averaging all voxels in each ROI. FC between each pair of ROIs was then evaluated using Pearson's correlation coefficients and the association of each FC and each trait scale was evaluated in the same way. The significance of these relationships was evaluated by the Bayes Factor (BF), which does not suffer from multiple testing issues (Beland et al., 2012) and prevents a great loss of power caused by family-wise error control. Results showed that the BFNES was related with strengthened inner temporal and temporal-prefrontal connectivity ($rs \geq .69$, $BFs \geq 34$). The SIAS was related with strengthened sensory/motor ($rs > .69$, $BFs > 41$) and semantic networks ($r = .72$, $BF = 68$), and weakened semantic integration network ($rs < -.69$, $BFs > 41$). Lastly, the SPS was related with strengthened emotional response ($r = .71$, $BF = 54$) and visual perception networks ($r = .69$, $BF = 34$), and weakened hub connectivity of the default-mode network ($rs < -.70$, $BFs > 53$). Interestingly, there was no overlap in FC alterations among the phenotypes and subtypes of social anxiety. These results suggest that differences between subtypes of SAD are reflective of dissociated neural dysfunction. Furthermore, these dysfunctions may be related to the occurrence of phenotypes caused by specific cognitive disruptions (e.g., perception, memory, and self-reference) independent of the trait of fear of negative evaluation.

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Poster

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Support: PRESTO Sakigake 237602

SRPBS 09010096

Kakenhi 26242087

Title: Amygdala activity in normal subjects induced by the inequity predicts their depressive tendency

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Abstract: During the last decade or two, mood disorders including major depression (MD) have become a big problem. It is known from many cohort-based studies that social stresses such as inequity is linked with MD, and that there is a significant sex difference in MD population. However, little is known about the neural substrates behind these key characteristics of MD. The aim of the current study is to link brain activity to the inequity, and depressive tendency and its sex difference. We conducted three behavioral and fMRI measurements: 1) Beck Depression Inventory –II (BDI-II) to evaluate depressive tendency 2) social value orientation (SVO) test to examine subjects' attitude toward distribution of resources (n =233, male: female = 126: 107), and 3) functional MRI (fMRI) for localizing the regions responsive to the inequity. Specifically, we asked subjects (n=98) to be a responder in the modified version of the ultimatum game and to decide whether to accept or reject an offered division. The inequity-induced responses were defined as the brain activities correlated with the absolute value of reward difference between proposers and participants($\neq$0, logarithmic scale) at the timing of presentation of offers. Behaviorally, in male subjects, the score distribution of BDI-II total score was located significantly higher (with a longer tail, $p = 0.037$) in prosocials (Pro, n = 86) than in

individualists (Ind, n = 40). In parallel, amygdala/hippocampus (amy/hippo) activation correlated with inequity was higher in Pro males than Ind males ($p < 0.001$, uncorrected; Pro: Ind = 32: 25). Furthermore, positive correlation between the amygdala activity and BDI-II total score was also found in Pro males ($p < 0.05$). We also conducted hierarchical clustering of BDI-II scores to characterize the high-BDI score population. We found not only that MD-suspects selected by the hierarchical clustering tended to have high scores in mental questions rather than physically-related questions, but also that those MD-suspects showed higher amy/hippo activity in correlation with the inequity ($p<0.001$). Thus, our result demonstrated the first neuroscientific evidence that links depression tendency in male prosocial subjects, and the amygdala/hippocampus sensitivity to the inequity, highlighting a potential applicability of brain activity to the inequity as a social brain marker for developing depression.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSF GRF to K.R.

Title: Language in social interaction modulates mentalizing networks

Authors: *K. RICE, K. VELNOSKEY, E. REDCAY
Univ. of Maryland, College Park, MD

Abstract: The second-person neuroscience movement urges social neuroscientists to adopt more ecologically valid and socially interactive paradigms, as opposed to traditional offline paradigms (e.g., listening to recorded sentences; Schilbach et al., 2013). Few studies, however, directly compare the neural correlates of processing the same human social stimuli in offline versus interactive contexts. Thus, it is unclear whether interaction alone alters the neural correlates of social cognition. We used a novel paradigm to examine whether a live, interactive context altered the neural correlates of speech processing. Thirty-one adults (13 males) completed an fMRI task in which they listened to audio in two conditions: Live and Not-Live. In the Live condition, participants listened to a social partner that they believed was speaking over a live audio feed. In the Not-Live condition, participants listened to recorded speech from another speaker.

Unbeknownst to participants, the Live segments were prerecorded, allowing for matched speech characteristics and linguistic content across conditions. The audio segments did not reference people or social situations (e.g., "There are two books"). A subset of adults (n=23) also completed an explicit mentalizing localizer task (Dodell-Feder et al., 2011) from which we created individual regions of interest (ROIs) corresponding to areas that were selective for thinking about others' mental states. Whole-brain analysis comparing Live to Not-Live audio revealed increased activity in regions often associated with mentalizing (e.g., dMPFC, left TPJ). The ROI analysis also revealed significantly increased activity for the Live vs. Not-Live audio contrast in functionally-defined regions implicated in explicit mentalizing (i.e., bilateral TPJ, dMPFC, precuneus). As different speakers were used for the Live and Not-Live conditions, we investigated whether differences between conditions could be due to speaker familiarity or differences in speaker characteristics. Separate behavioral testing (N = 40) indicated that, when all audio was presented in a recorded context, Live and Not-Live speakers were rated as equally direct, friendly and engaging (ps > .10). Thus, the differential neural effects of the Live and Not-Live conditions appear to be solely attributable to the perceived live interaction. These findings provide evidence that real-time social interactions alter the neural processing of language in regions associated with mentalizing, even without direct reference to mental states. These results have implications for understanding the real-world social brain, especially for social disorders like autism.

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Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant Z01-MH002813

Title: Brain regions with baseline group differences in BOLD signal & correlations with scopolamine antidepressant response

Authors: *J. S. ELLIS, A. C. NUGENT, C. A. ZARATE, Jr., M. L. FUREY
NIH, Bethesda, MD

Abstract: Previous findings indicate that baseline BOLD signal in middle occipital cortex correlates with the magnitude of antidepressant response to scopolamine during an emotion-specific working memory (WM) task. Using the same subjects and WM task, this analysis

compared patients and healthy volunteers on differences in BOLD signal between explicit and implicit emotion processing conditions, and examined the correlation between magnitude of antidepressant response to scopolamine and the differences in BOLD signal between conditions. Patients with major depression (n=13) and healthy volunteers (n=21) completed a pre-treatment fMRI scan. In separate conditions, subjects were instructed to match emotional face stimuli on the basis of either emotion or identity. Echo planar imaging data was acquired in a GE 3T scanner (TE= 23, TR= 2.5, slices=35). Subjects then participated in a double-blind, placebo-controlled, crossover clinical trial with scopolamine (4 μg/kg; iv). The Montgomery-Asberg Depression Rating Scale (MADRS) measured depression severity before and after scopolamine. Scans were preprocessed using AFNI, and a multiple linear regression was used to estimate BOLD response for each task condition. The difference in BOLD response for the two WM conditions (delta) was calculated, and a one-sample t-test was used to identify brain regions where the delta response differed between the two groups. A correlation analysis also was conducted between the delta and the magnitude of scopolamine treatment response in the patient group (change in MADRS) (voxel $p< 0.005$, whole brain corrected at $p< 0.05$). Group comparisons show that delta is significantly greater in patients than controls, indicating patients have a larger BOLD response in a network of regions during emotion relative to identity conditions, including anterior cingulate and bilateral insula extending into superior temporal gyrus, as well as middle frontal gyrus and dorsal parietal. Further, the magnitude of difference in BOLD signal between explicit and implicit conditions correlated with scopolamine treatment response in regions that overlap with those identified in the group comparison, including anterior cingulate and bilateral insula, as well as in regions not seen in the group analysis, including posterior cingulate cortex. These results suggest the same brain regions that show elevated BOLD response to explicit WM in patients relative to controls also show correlations with subsequent scopolamine response. The extent of this BOLD response difference between task conditions may provide a useful biomarker for identifying patients who may have a greater response to scopolamine.

Disclosures: **J.S. Ellis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-author has an intellectual property patent on scopolamine. **A.C. Nugent:** None. **C.A. Zarate:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending on the use of ketamine for depression. **M.L. Furey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent pending on the use of scopolamine for depression.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: Economic and Social Research Council (ESRC) Small Research Grant ES/J006793/1

Title: Anterior medial prefrontal cortex implements social priming of mimicry

Authors: *A. F. HAMILTON[1], Y. WANG[2]
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Abstract: The neural and cognitive mechanisms by which primed constructs can impact on social behavior are poorly understood. In the present study, we used fMRI to explore how scrambled sentence priming can impact on mimicry behavior. Sentences involving pro/antisocial events from a 1st/3rd person point of view were presented in short blocks, followed by a reaction-time assessment of mimicry. Behavioural results showed that both prosociality and viewpoint impact on mimicry, and fMRI analysis showed this effect is implemented by anterior medial prefrontal cortex (amPFC). We suggest that social primes may subtly modulate processing in amPFC in a manner linked to later behavior, and that this same region also implements the top-down control of mimicry responses. This priming may be linked to processing of self-schemas in amPFC. Our findings demonstrate how social priming can be studied with fMRI, and have important implications for our understanding of the underlying mechanisms of prime-to-behavior effects as well as for current theories in social psychology.

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Poster

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JSPS Excellent Young Researcher Overseas Visiting Program

Dr. Mortimer and Theresa Sackler Foundation

Title: Inferior parietal cortex mediates social influence on motor output

Authors: *M. YOSHIE[1,2], Y. NAGAI[2], H. D. CRITCHLEY[2,3], N. A. HARRISON[2,3]
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Abstract: The skilled motor performance of even the most talented athlete can be devastatingly impaired under the spotlight of social evaluation. Social-evaluative threat (SET) triggered by the awareness of potentially negative evaluation by critical witnesses can increase tension in muscles opposing the desired action, impairing fine motor control. Although the adverse effects of SET on motor performance are widely recognized, how this is mediated within the brain remains poorly understood. Here we emulated real-time social monitoring of participants' performance on a skilled motor task to manipulate SET naturalistically during functional magnetic resonance imaging (fMRI). Each participant was presented with video footage of two observers who appeared to be closely evaluating the participant's own performance (SET condition) or that of another participant (control condition). At the same time the participant performed a feedback-occluded isometric grip task at either 5 % or 10 % of their maximal voluntary contraction (MVC). These two levels of physical demand were used to distinguish brain activity underlying motor from non-motor effects of SET. The effectiveness of SET manipulation was evidenced by significantly higher self-rated state anxiety in the SET, compared to the control, condition. As anticipated, SET affected participants' motor output in a task-dependent manner: importantly, it had no effect on isometric grip force during the low-demand 5 %MVC task, yet significantly increased grip force during the more demanding 10 %MVC task. We were thus able to isolate activity underlying non-motor effects of SET by identifying brain regions commonly activated by SET in both the 5 % and 10 %MVC tasks. This conjunction analysis revealed significantly enhanced activity within the right posterior superior temporal sulcus. On the other hand, observed motor effects of SET accompanied focal reductions in activity within inferior parietal and inferior frontal cortices. Deactivation of the left inferior parietal cortex (IPC), encompassing the rostral inferior parietal lobule and anterior intraparietal sulcus, further predicted within- and between-individual differences in SET-evoked changes in grip force. These results indicate that the IPC plays a key role in mediating the effect of SET on motor output.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Neurosystem Lab. Universidad de Chile

Title: Mechanisms of autonomic regulation during social cognition task

Authors: *G. VARAS, E. BRUNETTI, P. E. MALDONADO
Univ. De Chile, Santiago, Chile

Abstract: The perception, interpretation and generation of responses to the intention and behaviors of others are known as social cognition (3). The recognition of facial expressions and the ability to infer the likely mental states of other people are an important feature of social cognition, this ability is called Theory of Mind. The emotions that humans experience while interacting with their environment are associated with varying degrees of physiological arousal (2). A key system involved in the generation of this physiological arousal is the autonomic nervous system (ANS). Heart rate variability (HRV) analysis is emerging as an objective measure of regulated emotional responding, and functions related to social cognition and Theory of mind. The polyvagal theory and the Neuro visceral integration model proposes that the ANS, through vagal tone activity and activity of the prefrontal cortex, improves the interactions of a subject with their environments through an inhibitory effect on the sino-atrial node (pacemarker) (1).There is evidence that, at rest, subjects with spinal cord injury (SCI) have a predominance of sympathetic autonomic activity which correlates with low HRV (4). Our hypothesis proposes that this type of basal activity of the ANS decrease autonomic flexibility that has been described as favorable for social cognition tasks. We measured HRV, as autonomic marker, in 18 healthy subjects and 10 subjects with SCI, diagnosed with paraplegia, who were pursuing a period of adaptation and socio-labor integration. A 5 min. quiet sitting period at the beginning of the assessment was used to collect baseline HRV. Than HRV was measured during performance of the The Reading the Mind in the Eyes Test (RMET), which assesses the affective component of the theory of mind. Based on our results it was observed that the group of subjects with SCI had a worse performance in the test ($p=0.001$), a significantly lower level of security on responses compared with the group of healthy people ($p=0.002$), lower HRV at rest($p=0.005$), and a smaller increase in the HRV during the task relative to the baseline condition (0.007). These results suggest that there be alterations in social cognition, in subjects with SCI, diagnosed with paraplegia, who were pursuing a period of adaptation and socio-labor integration. Our results also confirm a positive correlation between limitations in autonomic flexibility and worse performance in social cognition tasks.

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Poster

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Support: NIMH Conte Center Grant on the neurobiology of social decision-making

JSPS postdoctoral fellowship for research abroad

Title: Neural mechanisms underlying human consensus decision-making

Authors: ***S. SUZUKI**[1,4,5], R. ADACHI[2], S. DUNNE[3], P. BOSSAERTS[6], J. P. O'DOHERTY[2]
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Abstract: Consensus formation is a hallmark of human and animal societies. Collective decision-making potentially offers various advantages which include a reduction of risk from predators, as well as an enhancement of decision accuracy. Yet, little is known about how human consensus arises from interactions among group members, while the underlying neural mechanisms of this process are as yet unclear. To address the issue, we developed a novel fMRI experimental paradigm in which for each participant inside the scanner (20 participants were scanned in total), 5 other individuals were present outside. In our main experiment, all six individuals (per session) simultaneously made choices between two snack/trinket items repeatedly; and if they chose the same one (i.e., consensus) they were rewarded with the item. We also conducted a separate control experiment in which participants were asked to reach consensus with a computer algorithm. After performing computational analyses on behavioral and fMRI data in the main experiment, we found that participants' choices were guided by their own preferences, as well as the group members' previous choices. Furthermore, participants also kept track of the likelihood of consensus on each of the two items, determined through fitting a Bayesian-learning algorithm that takes into account the degree to which others' conform to the majority's choice. These different variables were encoded in different brain structures, with ventromedial prefrontal cortex (vmPFC) representing the participant's own preferences, the posterior superior temporal sulcus (pSTS) tracking the group members' prior choices, and

intraparietal sulcus (IPS) tracking the likelihood of consensus. Furthermore, while both vmPFC and IPS were present in both the social consensus and the computer control condition, pSTS appeared to be selectively recruited only in the social but not the control condition. These findings suggest that human consensus decision making may be mediated by the interaction of at least two distinct mechanisms: domain general processes associated with stimulus valuation and learning about the environment, as well as a social-specific brain system that learns predictions about other people's likely behavior.

Disclosures: S. Suzuki: None. **R. Adachi:** None. **S. Dunne:** None. **P. Bossaerts:** None. **J.P. O'Doherty:** None.

Poster

460. Human Social Cognition I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 460.20/TT7

Topic: F.01. Human Cognition and Behavior

Support: ARC Discovery Project: DP130100559

Title: Individual differences in affective and cognitive empathy are associated with differences in brain structure

Authors: *R. ERES[1], J. DECETY[2], W. R. LOUIS[1], P. MOLENBERGHS[1]
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Abstract: Understanding empathy from a neuroscience perspective often involves measuring BOLD responses to a range of empathy invoking tasks. Fan and colleagues (2011) recently conducted a meta-analysis of 40 fMRI studies that implicated distinguishable brain regions associated with the different components of empathy. They showed that tasks related to affective empathy (vicariously sharing others emotions) invoked responses from bilateral insula, whereas tasks related to cognitive empathy (reasoning about others' emotional states) invoked peak activity in the dorsomedial prefrontal cortex (dmPFC) and adjacent midcingulate cortex (MCC). Using this information, we predicted that these behavioural and functional differences were undermined by individual differences in brain morphometry. To test this hypothesis, we performed a voxel based morphometry (VBM) analysis. Scores on the questionnaire of cognitive and affective empathy (QCAE) were used as regressors to test the hypothesis that greater

affective empathy scores would be subserved by greater grey matter density in bilateral insula and higher scores on the cognitive empathy measure would be associated with greater grey matter density in the dmPFC and adjacent MCC. One hundred and seventy-six human participants completed the QCAE and underwent MRI with a high-density structure T1 scanning protocol. A factor analysis on QCAE scores confirmed the reliability and validity of the measure by presenting two clearly defined factors of cognitive empathy and affective empathy. VBM analyses revealed, in line with hypotheses, that higher scores on affective empathy, were associated with higher grey matter density in bilateral insula. A similar pattern of results was found for cognitive empathy, where increased grey matter density in the dmPFC and adjacent MCC were associated with higher ratings of cognitive empathy. Here we have shown individual differences in empathy to be subserved by differences in brain morphometry.

Disclosures: R. Eres: None. **J. Decety:** None. **W.R. Louis:** None. **P. Molenberghs:** None.

Poster

460. Human Social Cognition I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 460.21/TT8

Topic: F.01. Human Cognition and Behavior

Support: Department of Education H133F130009

Title: Ventromedial prefrontal cortex lesions reduce perceived radicalism of political beliefs

Authors: *I. CRISTOFORI[1], V. VIOLA[2], A. CHAU[1], W. ZHONG[1], F. KRUEGER[3], G. ZAMBONI[4], J. GRAFMAN[1]
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Abstract: Given the determinant role of ventromedial prefrontal cortex (vmPFC) in valuation, we examined whether its lesions also modulate judgments of political beliefs. Patients with penetrating traumatic brain injury (pTBI; N=102) and healthy controls (HC; N=31) were tested on the Political Belief Task, where they rated 75 statements expressing political opinions related to welfare, economy, political involvement, civil rights, and war & security. Each statement was rated for level of agreement and along three dimensions: radicalism, individualism, and conservatism. Voxel-based lesion-symptom mapping (VLSM) analysis showed that lower scores

for the radicalism dimension (i.e., statements were rated as less radical than the norms) were associated with lesions in bilateral vmPFC. After dividing the pTBI patients into three groups according to lesion location (i.e., vmPFC, dorsolateral prefrontal cortex (dlPFC) and parietal cortex), we found that the vmPFC, but not the dlPFC, group had lowered radicalism scores compared to parietal and HC groups. These findings highlight the crucial role of the vmPFC in appropriately valuing political opinions and could help explain certain inappropriate social behaviors observed in patients with vmPFC lesions.

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Poster

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Program#/Poster#: 460.22/TT9

Topic: F.01. Human Cognition and Behavior

Support: UMD DRI

UMD MNC

Title: White matter integrity in the uncinate fasciculus correlates with face-based theory of mind abilities in 4 but not 6 year olds

Authors: *L. C. ANDERSON, K. RICE, E. REDCAY
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Abstract: Successful social interaction requires the ability to infer what others are thinking, or Theory of Mind (ToM; Premack & Woodruff, 1978). ToM behaviors and their supporting brain systems undergo significant development in early childhood but little research has examined the relation between brain and behavioral development. One aspect of ToM critical to social interaction is inferring what other people are thinking or feeling based on their facial expression. This ability correlates with amygdala volume in early childhood (Rice et al., 2014) and is impaired in patients with early (but not late) amygdala lesions (Shaw et al., 2004). These data suggest an important role of the amygdala in the development of face-based ToM, but no study has yet examined the relation between amygdala connectivity and social cognitive development in early childhood. This study aimed to determine if indices of structural connectivity (e.g.

fractional anisotropy) in white matter tracts are related to face-based ToM in typically developing 4 and 6 year olds. We focused on the uncinate fasciculus (UF) based on its proximity to the amygdala and previously studied role in social cognition (Von Der Heide et al., 2013). Structural and diffusion-weighted data were collected from 49 children: 20 four year olds and 29 six year olds. Diffusion-weighted images were visually inspected for motion artifacts and processed using FreeSurfer's TRACULA (Yendiki et al., 2011)**.** TRACULA uses Bayesian statistics to reconstruct probabilistic distributions of white matter tracts from participants' native diffusion images using anatomical priors and has been successfully used with child data (Ghosh et al., 2010; Yendiki et al., 2013). We used multiple regression analysis to predict face-based ToM (Simplified Eye-Reading Test; SERT; Peterson & Slaughter, 2009) from Age (in months) and UF fractional anisotropy. The model for Left UF, but not Right UF, was statistically significant, $F(3,45) = 3.00$, $R^2 = 0.18$, $p = 0.04$, and Age, Left UF, and the Age x Left UF interaction all had significant partial effects in the full model ($p < 0.05$). Follow-up analyses suggest that while 4 year olds show a positive relationship between Left UF and SERT scores, 6 year olds do not. Importantly, an analysis with an adult sample ($N = 20$) showed the same pattern as the 6 year olds—no relationship between Left or Right UF and face-based ToM abilities. In the child sample, identical analyses with a control tract were nonsignificant, providing evidence for the specificity of the current results. Findings suggest that at age 4, the structural integrity of the UF predicts children's face-based mentalizing abilities, but by age 6, this is no longer true.

Disclosures: L.C. Anderson: None. **K. Rice:** None. **E. Redcay:** None.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: MEXT KAKENHI Grant Number 25730172

Title: Neural mechanisms underlying changes in preference for visual motor stimuli after exposure with imitation and observation

Authors: *Y. OGATA, T. HANAKAWA
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Abstract: Recently, many psychologists have studied about a phenomenon called "mere exposure effect (MEE)", which is repeated exposure of a stimulus object enhances an attraction to the object. However, it remains unclear whether MEE occurs in the case of repetitive

visuomotor association and, if so, what the neural basis of such a phenomenon is. In the present study, we aimed at clarifying the effects of repeatedly performed or observed actions on preference evaluation for visually presented action stimuli (visuomotor MEE) and identifying brain regions involved in the visuomotor MEE by using fMRI. Twenty-four healthy subjects first participated in a pre-rating session with a set of visual stimuli showing hand-signs (finger alphabets). We used visual analogue scaling for obtaining a preference rating (PR) based on a like-dislike scale and also an easiness rating (ER) based on an easy-difficult scale. Next, they were repeatedly presented with the hand-sign stimuli (10 times), and instructed to imitate or merely observe them (exposure session). As control stimuli, we prepared a set of hand-sign stimuli used only for PR and ER (no exposure). After the exposure to the stimuli, participants underwent a post-rating session for PR and ER of all of the stimuli. We measured brain activity during all of sessions using fMRI. The behavioral data showed that increases in PR for the imitated stimuli were significantly larger than those for the control stimuli ($p<.01$). PR for the observed stimuli also showed a marginally significant increase ($p=.09$). However, the difference in ER between the pre and post-rating sessions was not significantly affected by the exposure. These results suggest that not only merely repeated observations but also repeated imitations yielded MEE. An analysis of brain activity during the pre- and post-rating sessions revealed activation in posterior cingulate cortex (PCC), right caudate nucleus, bilateral orbitofrontal cortex (OFC) and left inferior parietal lobule (IPL). In these areas, PR for the imitated stimuli induced greater activity in the post-PR than the pre-PR; furthermore, this difference was greater than the changes of PR-related activity (post-PR minus pre-PR) for the control stimuli ($p<.001$, uncorrected). Additionally, we found that signals in IPL decreased in correlation with the repetition of imitation and that the deactivation correlated with the effect size of MEE. Our results suggest that repeated motor imitation elicits MEE for visuomotor-associated stimuli and that the value-related network (PCC, OFC and caudate) and the mirror neuron system (IPL) are involved in the phenomenon of visuomotor MEE.

Disclosures: **Y. Ogata:** None. **T. Hanakawa:** None.

Poster

460. Human Social Cognition I

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Program#/Poster#: 460.24/TT11

Topic: F.01. Human Cognition and Behavior

Title: Dual logic and cerebral coordinate for reciprocal social interaction

Authors: *R. LEE
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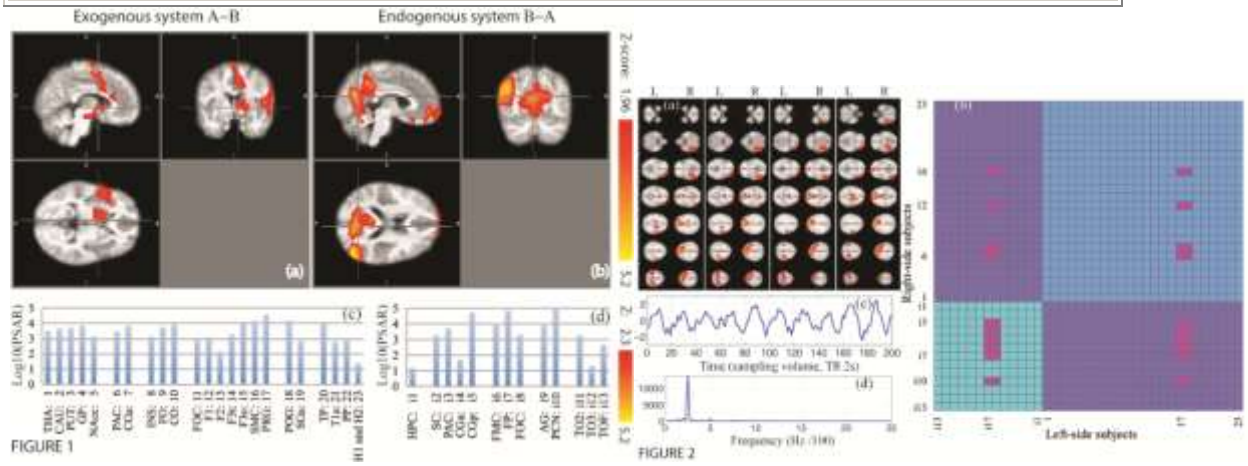
Abstract: Reciprocal interaction (RI) in eye contact can only be implicitly observed by dyadic fMRI (dfMRI) (1), because RI and non-RI are entwined. A dual logic (2) is developed to deductively derive explicit RI. Data processing based on the deduction yields a cerebral coordinate (CC) in which parallel reciprocal processes during gazing can be quantified. 19 pairs participate in the dfMRI experiment, where tasks A and B are described in Ref. (1). Group GLM and their paired comparison, A-B and B-A, are calculated by FSL. Based on dual logic (2), A-B and B-A are dichotomous dual systems for RI (Tab. 1): The A-B is the exogenous RI, and the B-A is the superposition of endogenous RI and the default-mode (u=0, v=0) (Fig. 1). The masks and atlas labels of the A-B and B-A subserve the function of CC for RI. The parallel neural processes during gazing are embedded in the dyadic data of task A. They can be decomposed by ICA. Group ICA from the 19 pairs data yields 35 independent components (IC). Projecting all ICs on the CC, only RI related ICs are selected. One of them is in Fig. 2. (1) Lee, R, et al (2012). MRM, v. 68, p. 1087 (2) Lee, R, in review

TABLE 1: Truth-table of the dual logic deduction

	reciprocal state	non-reciprocal state	A-B response	B-A response
Exogenous system	x	y	$x \wedge \neg y$	0
	0	0	0	0
	0	1	0	0
	1	0	1	0
	1	1	0	0
Endogenous system	u	v		$\neg(u \wedge v) \wedge \neg v$
	0	0		i
	0	i		0
	i	0		i
	i	i		0

"1" and "0" are true in exogenous and endogenous system respectively



Disclosures: R. Lee: None.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R37AG009253

Yale University FAS Imaging Fund

Title: Distinct neural networks support the mere ownership effect under different motivational contexts

Authors: K. KIM, *M. K. JOHNSON
Dept. of Psychology, Yale Univ., New Haven, CT

Abstract: The “mere ownership effect” refers to individuals’ tendency to evaluate objects they own more favorably than identical objects they do not own (Beggan, 1992). There are numerous behavioral demonstrations of this self-positivity bias during the evaluation of self-associated objects, but the neural mechanisms underlying its expression have only recently begun to be investigated (e.g., Kim & Johnson, 2010). The goal of the present study was to identify the

neurobiological expression of the mere ownership effect and to assess the potential influence of motivational context on the neural expression. During fMRI scanning, participants made evaluations (i.e., preference ratings) of a number of consumer objects (e.g., clothing, stationary, electronic articles) after ownership had been assigned. Motivational context was manipulated by using a self-esteem threat procedure involving bogus performance feedback on an ostensibly unrelated task. In the *absence* of self-esteem threat, the mere ownership effect (i.e., increased preference for objects from before to after ownership acquisition) was associated with the engagement of brain regions implicated in processing personal/affective significance and self-relevancy (ventromedial prefrontal cortex [vMPFC], medial orbitofrontal cortex [mOFC], and ventral anterior cingulate cortex [vACC]). In contrast, in the *presence* of self-esteem threat, the mere ownership effect was associated with brain regions implicated in selective/inhibitory cognitive control processes (inferior frontal gyrus [IFG], middle frontal gyrus [MFG], and lateral orbitofrontal cortex [lOFC]). These findings indicate that depending on motivational context, different neural processes (and thus likely different psychological mechanisms) support the behavioral expression of self-positivity biases directed toward objects that are associated with the self.

Disclosures: K. Kim: None. **M.K. Johnson:** None.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: The James S. McDonnell Foundation

The Swedish Research Council

Söderberska Stiftelsen

Title: Dissociating the neural substrates of self-awareness and perceptual-awareness

Authors: *P. TACIKOWSKI, C. C. BERGER, H. H. EHRSSON
Neurosci., Karolinska Inst., Stockholm, Sweden

Abstract: Perceptual-awareness refers to the integration of sensory information and to making it available for multiple cognitive functions, including attention, memory, etc. Self-awareness, in

turn, is a special case of perceptual-awareness, and occurs when the information being processed relates to the self. The aim of this fMRI study was to test whether the two processes have distinct neural correlates. We hypothesized that self-awareness would engage mainly medial fronto-parietal regions, whereas perceptual-awareness would be associated with activations in the lateral fronto-parietal regions. Subjects (n = 26) saw self- or other-related stimuli (a name, surname, etc.) and their task was to decide whether the information relates to them or not. In half of the trials the visual masking procedure was used (low-awareness context) and in the other half no masking was used (high-awareness context). In this paradigm self-awareness was operationalized as a significant interaction effect (stronger self-specific BOLD adaptation only in the high-awareness context) and perceptual-awareness as a significant main effect (stronger BOLD adaptation in the high- than in the low-awareness context, regardless of the self- or other-related content). In addition, the paradigm allowed identifying neural correlates of automatic self-related information processing (stronger BOLD adaptation during processing of self- than other-related information, regardless of the level of awareness). Consistently with our predictions, activations specific to self-awareness were found in the medial prefrontal ($t = 4.81$, $p_{corr} = 0.008$) and the posterior cingulate cortices ($t = 3.55$, $p_{corr} = 0.049$), whereas perceptual-awareness was related to activations in the left precentral ($t = 3.85$, $p_{corr} = 0.04$) and left intraparietal sulci ($t = 4.33$, $p_{corr} = 0.039$). In addition, the automatic processing of self-related information engaged the left and right inferior temporal cortices ($t = 3.76$, $p_{corr} = 0.028$ and $t = 4.08$, $p_{corr} = 0.038$, respectively). Next, we examined the intrinsic connectivity between regions of the perceptual- and self-awareness networks using resting-state fMRI from the same group of subjects. We found that low-frequency BOLD fluctuations were positively correlated within each of the two networks and negatively correlated between the two networks. These results further support our fMRI findings and suggest that the functional dissociation between perceptual- and self-awareness networks goes beyond our specific behavioral paradigm. Together, this study presents the first evidence on separate brain mechanisms involved in perceptual- and self-awareness.

Disclosures: **P. Tacikowski:** None. **C.C. Berger:** None. **H.H. Ehrsson:** None.

Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: SCOPE

Title: Choose the right clothes: An fMRI study of the integration of social and participant's situation

Authors: *K. OBA, M. SUGIURA, S. HANAWA, M. SUZUKI, H. JEONG, Y. SASAKI, Y. KOTOZAKI, T. NOZAWA, S. NAKAGAWA, T. KIKUCHI, R. KAWASHIMA
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Abstract: Considerable studies have shown the neural substrates of the recognition of social situation. In the actual social recognition, however, it is also essential to consider the participant's situation, for instance, when we recommend appropriate clothes for others who will participate in a specific social situation. Thus far, there have been no studies considering the process of taking participants situation into account in the understanding of social situation. Specifically, this process is to find the participant's appropriate role in the situation (role processing). When one cannot easily find such a role, this process is cognitively demanding due to the necessity of exploring an appropriate role from atypical candidates (role exploration). Here, we aimed to investigate the neural bases of the "role processing" and "role exploration" in the recognition of social situation. Right-handed healthy undergraduate/graduate students (n=28, male=17, female=11, 20.4±1.7 years old) participated in this study. All subjects gave written informed consent prior to the experiment. In this study, we created a "recommendation task", in which subjects were asked to recommend an appropriate clothes to the other person (participant) who will participate in the specific social situations (e.g., wedding party and academic conference). We measured brain activity during subjects recognized the social situations using functional magnetic resonance imaging. There were following conditions: (A) participant situation(+)/social situation(+), which required the consideration of participant's situation to recommend an appropriate clothes, (B) participant situation(+)/social situation(+)/cognitive demand(+), which required some effort to recommend an appropriate clothes to the situation, and (C) participant situation(-)/social situation(+), which required social situation recognition without considering participant situation. In the comparison of the condition A versus C ("role processing"), we found greater activity in the premotor cortex (PM) and inferior parietal lobule (IPL) ($p<0.001$ uncorrected), which are associated with motor simulation (Jeannerod, 2001, NeuroImage). In the comparison of the condition B versus A ("role exploration"), we found greater activity in the medial orbitofrontal cortex (mOFC) ($p<0.001$ uncorrected), associated with the prediction of possible alternatives in the prerecognition process (Bar, 2007, Trends Cogn Sci). The results suggest the involvement of sensorimotor and predictive processes in the recognition of social situation in practical contexts.

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Poster

460. Human Social Cognition I

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Support: the Consumer Perception Study grant (R1107731) from Kimberly-Clark Worldwide, Inc.

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Title: Neural origins of the fear of negative evaluation by others: An fMRI study on incontinence

Authors: D. JUNG[1], K. KIM[2], J. LEE[1], M. KIM[2], S. LEE[3], W. SOHN[3], *H. KIM[2]
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Abstract: An excessive fear of negative evaluations by others appears to be the primary source of the anxiety prevalent among social phobic individuals. Incontinent people often suffer from irrationally heightened social anxiety and negative self-images due to their urinary syndromes, which often lead to difficulty in maintaining social relationships with others. The present study was aimed to examine the neural correlates of the fear of symptom-related negative evaluation by others in incontinent people. Inside fMRI machine, thirty female participants (14 control and 16 incontinent people) performed self-referential task where they evaluated how well a series of adjectives describe their personality traits from either a first- or a third-person (i.e., husband) perspective. Participants evaluated a total of 90 adjectives, which were classified into 3 subcategories: positive, symptom-related negative, and symptom-unrelated negative personality trait words. Consistent with a large body of previous literature, participants were more likely to consider positive and negative traits as more and less self-descriptive, respectively, although no significant difference was observed between the control and the incontinent groups in this behavioral pattern. The fMRI data revealed that, compared to the control group, the incontinent

group showed greater activities in left insula and medial prefrontal cortex during the third-person and first-person perspective condition, only while evaluating symptom-related negative adjectives. We also performed a psychophysiological interaction (PPI) analysis, to identify the brain regions where their communications with the left insula change as the participants shift perspectives. This analysis revealed that the medial prefrontal cortex showed stronger functional connectivity with the left insula during the husband vs. self condition, and this pattern was observed in the incontinent but not the control group. The present study suggests that the left insula working together with the medial prefrontal cortex may be at the core of excessive fears of negative evaluation by others often observed in incontinent people. We also believe that the present findings can be extended to the understanding of the pathophysiology of social phobia, one of the most commonly diagnosed anxiety in many societies.

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Poster

460. Human Social Cognition I

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: Direction Générale de l'Armement - Post-doctoral Grant #2013.60.0020

Academy of Finland - Grant #131483, #263800

European Research Council - Advanced Grant #232946

Title: Modulation of rolandic beta-band oscillations during joint action

Authors: M. MÉNORET[1,2], G. ZHOU[1], M. BOURGUIGNON[1], *R. K. HARI[1]
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Abstract: Coordination in joint actions may require representing both own and the partner's actions. To improve our knowledge of the neural basis of joint action and its dynamics, we recorded whole-scalp MEG from 13 subjects while they were involved in joint action with the experimenter. The MEG participant was grasping and lifting a pen and the experimenter was taking the pen from him/her to draw a line. In a control condition, the participant was similarly lifting the pen but the experimenter was taking another pen to draw a line. Each trial lasted 8 s, and the conditions were presented in randomized order. The instruction, informing about the condition, was displayed for 2 s, whereafter a tone served as a Go signal. Participants were asked to fixate a cross throughout the whole trial and to place their hand back to the initial position after the movement. MEG was recorded from the participant, and accelerometer signals were obtained from both the participant's and the experimenter's index fingers. Movement onset times, movement durations and the level of rolandic beta-band oscillations (15-25 Hz) were analyzed and compared for both conditions. The movement onset times did not differ between the conditions; and the movements lasted on average 3.6-4.9 s. However, despite instructions to perform the movements at the same pace and as similarly as possible between the two conditions, participants' movement was on average 21% shorter in the joint-action than control condition whereas the experimenters' movement was on average 9% longer in the joint-action than the control condition. Modulation of the rolandic rhythms was assessed during the total duration (2 + 8 s) of the trials. The beta oscillations were suppressed over the left parietal region from the onset of the instruction and during movement execution, similarly during both conditions, and a strong beta rebound occurred after the movement. Beta rebound was assessed by time-locking the analysis to the participant's movement offset and using as the baseline the beta level during the movement, from 3 to 1 s before movement offset. The rebound was 19 ± 5% ($p < 0.001$) weaker during the joint-action than control condition. The observed difference between the conditions agrees with a different recruitment of the motor system during joint action and when the same movement is done individually. The weaker beta rebound during the joint action might be associated with the representation of the partner's action. However, future studies need to specify the effects of both participants' movement durations and velocities on the observed difference.

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Poster

460. Human Social Cognition I

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Topic: F.01. Human Cognition and Behavior

Support: NYU Abu Dhabi internal research grant

Title: Your brain on social robots: anthropomorphism and the 'uncanny valley' effect investigated with functional MRI

Authors: *Y. WANG[1,2], S. QUADFLIEG[2]
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Abstract: Mind attribution is important for understanding of social interaction. However, people don't only ascribe minds to humans, but also ascribe minds to non-humans such as robots. Perceiving robots can cause the 'uncanny valley' effect. According to this effect, encountering robots elicits increasingly positive feelings, the higher their human likeness until a level of realism is reached that makes perceivers uncomfortable (e.g. eeriness, revulsion). It has recently been proposed that this uncanny feeling results from a conflict between people's stereotypes on robots (i.e. 'machines don't possess a mind') and an inclination to anthropomorphize them (i.e. to attribute human minds to objects in order to understand/predict them). To test this claim, 26 participants were asked to view a series of photos depicting social interactions (e.g., shake hands, dance) between two humans (human-human interactions, HHI) or between a human and a robot (human-robot interactions, HRI). They also completed two functional localizer tasks that define mentalizing and person perception system. After the scanning, participants rated each of the photos (e.g. eeriness of the interaction, emotional capacity of the human/robot agents in the interaction). Exploratory whole-brain analysis and region of interest analysis both revealed that mentalizing and person perception system are strongly engaged when perceiving HRI (versus HHI). Specifically, mentalzing system such as dorsal and ventral medial prefrontal cortex (VMPFC) showed increased activations whereas temporal-parietal junction showed attenuated activations to HRI. Person perception system such as fusiform face area and middle occipital gyrus showed stronger activations to HRI, but the precise locations of these activations are spatially dissociable from those engaged in human face/body perception localizer. Further parametric analysis identified the neural correlates of eeriness rating in VMPFC. These results suggest that perceiving robots engages similar brain regions responsible for mentalizing humans, but different brain regions with those involving in recognizing humans face/body. VMPFC engages not only in antrhropomorphizing robots, but also in eeriness rating. We suggest that distinct activations in person perception system enable people to recognize robots and activate stereotype-based judgment of robots in VMPFC; however, when people are making sense of the HRI, anthropomorphizing robots conflicts with stereotypes of robots, which generates the feeling of eeriness in VMPFC. Therefore, our study suggests VMPFC as a neural basis for anthropomorphism and the 'uncanny valley' effect to robots.

Disclosures: Y. Wang: None. **S. Quadflieg:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 461.01/TT18

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH086591

Title: Small conductance Ca2+-activated K+ channel blockade in the lateral amygdala alters fear memory

Authors: *S. A. SANGUINETTI[1], R. W. STACKMAN, Jr[2]
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Abstract: Small conductance Ca2+-activated K+ (SK) channels have been shown to alter the encoding of spatial and non-spatial memory in the hippocampus by shaping glutamatergic postsynaptic potentials and modulating NMDA receptor-dependent synaptic plasticity. When activated, SK channels reduce hippocampal neuronal excitability and LTP. Similar SK channel properties have been demonstrated in lateral amygdala (LA) pyramidal neurons. Additionally, induction of synaptic plasticity and beta-adrenoreceptor activation in LA pyramidal neurons causes PKA-mediated internalization of SK channels from the postsynaptic density. Chronic activation of the amygdala through repetitive stressful stimuli can lead to excitatory synaptic strengthening that may create permanent hyper-excitability in its circuitry. This mechanism may contribute to a number of mood and anxiety disorders. The selective influence of SK channels in the LA on anxiety and fear conditioning are not known. The current study examined whether SK channel blockade by bee venom peptide, apamin, during a repetitive acute fear conditioning paradigm was sufficient to alter fear memory encoding and the resulting behavioral outcome. Naïve male C57BL/6J mice were surgically implanted with bilateral cannulae directed at the LA and received an intracranial microinfusion of apamin or saline prior to exposure to the elevated plus maze to determine whether SK blockade affected basal anxiety in mice. Mice were then given a 72-h rest period, then a second intra-LA micro infusion of apamin or saline prior to exposure to a 1 tone (CS) - foot shock (US) conditioning protocol. Tone fear memory was examined 24 h later. On the day after testing, mice received the same intra-LA treatment and were conditioned to a novel CS and context and tested on the novel fear memory strength 24 h later. Following the final fear memory test session, mice were separated into two groups: anxiety and locomotor responses were assessed in Group 1 mice in an open field immediately after the second fear conditioning test session, while Group 2 received the open field test after a 10 day delay. The findings indicate that intracranial LA microinfusions of apamin did not affect anxiety

prior to fear conditioning. Additionally, the findings suggest that simultaneous exposure to Pavlovian fear conditioning and SK channel blockade influence the subsequent sensitivity of mice to the impairing effects of apamin on fear conditioning. These data support the view that SK channels modulate synaptic plasticity that may underlie memory encoding in the lateral amygdala.

Disclosures: S.A. Sanguinetti: None. **R.W. Stackman:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH098506

Title: Fear conditioning leads to an increase in the number of PACAP neurons expressing cfos within the basolateral amygdala

Authors: *Y. HUANG[1], A. K. RAJBHANDARI[1,2], M. S. FANELOW[1,2], J. A. WASCHEK[1]
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Abstract: Recent studies indicate the involvement of the pituitary adenylyl cyclase-activating polypeptide (PACAP) and its G protein-coupled receptor PAC1 in the neural circuitry of stress. Post-traumatic stress disorder (PTSD) is reported to occur after exposure to intense traumatic experience and is conceptualized to involve inappropriate control over fear, and has recently been genetically linked to the PACAP/PAC1 signaling pathway. Thus, studying the involvement of the PACAP/PAC1 system in regulation of conditioned fear behaviors could help understand the neural mechanisms involved in PTSD. In this study, we sought to investigate the changes in the level of immediate early gene c-fos after fear conditioning in PACAP-EGFP mice (mice with Green Fluorescent Protein in PACAP-containing neurons). We hypothesized that acquisition of conditioned fear would alter neuronal activity in PACAP expressing neurons within the basolateral amygdala (BLA), a brain region crucial for mediating conditioned fear behaviors. A group of PACAP-EGFP mice were placed in a context in which they received a 0.65mA, 1-second foot shock after 4 minutes. After 6 consecutive days of this acquisition phase during which freezing levels reached an asymptotic level, half of the mice were tested in a novel context for changes in the level of fear while the other half were left in the home cage as controls. Ninety

minutes following the test in the novel context or home cage, all mice were perfused and their brains extracted. Using immunofluorescence procedures, positive c-fos and EGFP immunolabeling were analyzed and quantified in the BLA slices. Preliminary cell counting analysis revealed an increase in the number of PACAP neurons expressing c-fos in the test group than in the controls, indicating that fear expression may have altered the activity of PACAP neurons within the BLA. These results indicate that the PACAP expressing neurons in the BLA may be involved in the regulation of fear and that targeting this system may be important for understanding the neural circuitry underlying fear dysregulation in anxiety disorders including PTSD. Future studies are needed to understand the specific role of the PACAP/PAC1 system within fear circuitry.

Disclosures: Y. Huang: None. **J.A. Waschek:** None. **A.K. Rajbhandari:** None. **M.S. Fanelow:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant R01 MH062122

Title: Dissociating the relative contribution of NMDA receptors in the basal and lateral amygdala in supporting auditory and contextual fear learning

Authors: *S. R. STERLACE, M. M. FLESHER, N. NOCERA, M. S. FANSELOW
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Abstract: The basolateral amygdala complex, containing the lateral (LA) and basal (BA) nuclei, are critical for cued and contextual fear learning and memory formation through mechanisms that include *N*-methyl-D-aspartate receptor (NMDAR)-mediated synaptic plasticity. However, the relative contribution of NMDAR-mediated plasticity in the BA and LA is unknown because the pharmacological techniques previously used to implicate NMDAR have limited anatomical specificity. While lesion studies can be more anatomically precise, lesions affect far more than synaptic plasticity. To overcome these limitations we used Grin1^{flox} mice combined with stereotaxic delivery of an AAV2/1 virus coding for Cre-recombinase (Cre-GFP or GFP-only control) targeted at either the BA or LA. The virus will delete Grin1 from infected cells causing a

loss of GluN1, which is necessary for NMDAR. One-month after viral infusion, mice received fear conditioning, which consisted of five tone-shock pairings. Following training, mice were given both a context test and a tone test. Using an ANOVA separating groups by their nominal target infection region of either the LA or BA, we found that targeting the LA produced a deficit in tone fear, while targeting the BA produced a deficit in both tone and contextual fear. Since the viral constructs used were tagged with a green fluorescent protein, we were able to identify the spread of the virus and found that regardless of intended target there was often some infection of neighboring nuclei. Therefore, we performed immunohistochemistry to identify the amount of GluN1 per region, and used GluN1 levels as predictors in a linear regression model. Our model indicated that GluN1 levels significantly predicted time spent freezing during both the context and tone tests. Depletion of GluN1 in the BA was a strong predictor for a deficit in both contextual and auditory fear conditioning over and above the effect of GluN1 levels in the LA. The relationship between auditory fear conditioning and GluN1 levels in the LA fell just short of statistical significance. These results indicate that NMDARs in the BA are important for both auditory and contextual fear conditioning. They also illustrate how the combination of genetic and viral techniques, whose effectiveness can be directly assessed with immunohistochemistry and then correlated with behavior, allows far greater precision than previous methodologies.

Disclosures: S.R. Sterlace: None. **M.M. Flesher:** None. **N. Nocera:** None. **M.S. Fanselow:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Title: Stress-enhanced fear learning induces changes in AMPAR mediated excitatory currents, hyperpolarization-activated cation current (Ih), and K+ inward rectifier current (KIR) in principal neurons of the lateral amygdala

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Abstract: Fear, a healthy adaptive response, initiates defensive behavior to protect animals and humans from danger. Anxiety disorders, such as post-traumatic stress disorder (PTSD), can occur with inappropriately regulated fear. PTSD can occur as a response to a traumatic event with avoidant behavior, persistent re-experiencing of the trauma, increased arousal, and symptoms persisting longer than 1 month. A rodent model, stress-enhanced fear learning (SEFL), mimics several PTSD features, including resistance to extinction, and increased voluntary alcohol intake. Very little is known about the functional changes that take place in the amygdala of rats after SEFL. Previously we used Western blotting of basolateral amygdala tissue from male SEFL and control (CTL) rats, to show that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunit, glutamate receptor 1 (GluA1) was significantly increased after SEFL conditioning, without significant changes in GluA2, GluN1, or GluN2B subunits, suggestive of possible increases in AMPAR-mediated neurotransmission in the BLA. In this study we generated preliminary whole-cell patch-clamp recordings of AMPAR-mediated excitatory postsynaptic currents (EPSCs), hyperpolarization-activated mixed cation channel (Ih) current, and the K+-inward rectifier (KIR) current from principal (pyramidal) neurons in the lateral amygdala (LA) of male SEFL and CTL rats, approximately 2 weeks to 1 month after conditioning. We show that the paired-pulse ratio of evoked EPSCs was significantly increased (unpaired t-test; $p<0.05$) in SEFL (n=6) vs. CTL (n=7) rats, a trend of increased frequency of spontaneous EPSCs in SEFL (n=3) vs. CTL (n=4) rats, and a trend of increased frequency and amplitude of miniature EPSCs in SEFL (n=2) vs. CTL (n=3) rats. The Ih was significantly decreased (2-way repeated measures ANOVA; $F(1,25)=4.3$, $p=0.049$) in SEFL (n=11) vs. CTL (n=15) rats. The KIR measured at -130 mV was also significantly decreased (unpaired t-test; $p<0.05$) in SEFL (n=14) vs. CTL (n=16) rats. The number of action potentials evoked with a 200 pA (500 ms) depolarizing square-wave current pulses was significantly increased (Mann-Whitney rank sum test; $p=0.014$) in SEFL vs CTL rats. These data suggest that the SEFL procedure leads to long-term increases in the overall excitability of principal neurons in the LA.

Disclosures: E.M. Meyer: None. **J. Perusini:** None. **M.S. Fanselow:** None. **I. Spigelman:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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NIH/National Center for Research Resources base grant P51RR000165 to Yerkes National Primate Research Center

Title: Structural, functional and epigenetic responses to cue-specific olfactory fear extinction training

Authors: *F. G. MORRISON[1], B. G. DIAS[1], K. J. RESSLER[1,2]
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Abstract: Olfactory sensory neurons (OSNs) of the Main Olfactory Epithelium (MOE) provide a rich model to study the perception of external cues and the underlying mechanisms regulating structural plasticity within the olfactory system. Using the M71-LacZ mouse line (OSNs expressing the M71 odorant receptor can be visualized by LacZ immunohistochemistry (Vassali et al., 2002)), we have demonstrated an increased number of M71+ OSNs in the olfactory epithelium following olfactory fear conditioning to acetophenone (Jones et al., 2008), an odorant shown to specifically activate the M71 receptor. This increase was directly correlated with an increase in the M71+ glomerular cross-sectional area and volume within the olfactory bulbs. Notably, when animals receive the same odor-shock pairing to another odorant that does not activate M71, there are no detectable changes in the M71 neuron population or glomeruli. Functionally, mice exhibit enhanced freezing to the conditioned odor stimulus following olfactory fear conditioning. These previously published data indicate that the olfactory nervous system responds both structurally and functionally to olfactory fear, however, it is unknown whether previously acquired responses to the conditioned cue can be reversed by fear extinction. Using the M71-LacZ mouse line, we sought to determine whether the behavioral (increased freezing) and structural (increased number of M71+ OSNs and M71+ glomerular area) changes observed after olfactory fear conditioning may be reversed with extinction training. Using native chromatin immunoprecipitation (N-ChIP) protocols on the MOE, we investigate the dynamic alterations in permissive and repressive histone marks around the M71 gene locus following both olfactory fear acquisition and extinction. Male mice were trained to associate mild footshocks

with acetophenone using a session consisting of 5 odor-shock pairings (1 session/day for 3 days). 3 weeks after the last conditioning session, animals were handled only or exposed to an extinction session that involved the presentation of 30 acetophenone-only presentations (1 session/day for 3 days). 3 weeks after the last extinction session, animals were sacrificed. We demonstrate that extinction training specific to the conditioned odorant cue reverses the conditioning-associated increases in freezing and M71-specific OSN number and glomerular area. Furthermore, we demonstrate a dynamic regulation of histone marks around the M71 locus associated with both cue-specific fear learning acquisition and extinction. Our observations shed light on how the olfactory sensory system responds to extinction learning after fear conditioning.

Disclosures: F.G. Morrison: None. **B.G. Dias:** None. **K.J. Ressler:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Support: Howard Hughes Medical Institute

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NIH MH088467

Title: Identifying the translational profile of corticotropin releasing factor neurons in central amygdala during fear conditioning

Authors: *G. M. GAFFORD[1], B. G. DIAS[2], K. J. RESSLER[2,3]
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Abstract: An innovative technique that provides information about the translational status of mRNA transcripts present within specific cell types is Translating Ribosome Affinity Purification (TRAP, Heiman et al., 2008). A key feature of the TRAP protocol is that it measures translated RNA rather than more traditional approaches that measure the aggregate of all mRNA within the region of interest. To target translated RNA, the EGFP-L10a ribosomal transgene is expressed in a cell type of interest. Once expressed, the EGFP-L10a transgene can be extracted

yielding both the ribosome and associated mRNAs from the EGFP-L10a-expressing cell. The aim of the present study is to use the TRAP technique to determine the identity of RNA translated in the central amygdala (CeA) in specific cell types after fear conditioning and extinction. The CeA is of particular interest because of its involvement in fear memory and the diversity of its cellular makeup. The CeA expresses an array of neuropeptides including Corticotropin Releasing Factor (CRF). CRF is involved in initiation of endocrine, autonomic and behavioral responses to stressors. Dysfunction of the CRF system is implicated in a variety of mood and anxiety disorders and the RNA translated by these neurons during fear conditioning and extinction is not known. To investigate the translational profile of CRF neurons during fear behavior in the CeA, we created a CMV promoter-floxed stop-EGFP-L10a construct (CMV-GFP-L10a). We show that the CMV GFP L10a construct expresses in HEK cell ribosomes in a Cre dependent manner. Real time polymerase chain reaction data shows that co-transfection of HEK 293 cells with the CMV GFP L10a construct and Cre recombinase increases expression of ribosomal RNA compared to HEK 293 cells transfected with the CMV GFP L10a construct alone. Utilizing transgenic mouse lines infected with the CMV-GFP-L10a virus, ongoing studies are investigating the translational profile of CRF neurons in the CeA after fear conditioning.

Disclosures: **G.M. Gafford:** None. **B.G. Dias:** None. **K.J. Ressler:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Burroughs Wellcome Foundation

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Title: Voxel-based morphometry analysis of auditory fear conditioning in mice with concurrent confocal analysis of structural changes at the cellular level

Authors: ***O. P. KEIFER, JR**[1,5,2], R. C. HURT[2,5], D. A. GUTMAN[3], S. D. KEILHOLZ[6], S. L. GOURLEY[4,5], K. J. RESSLER[2,7,5]

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Abstract: Auditory fear conditioning (AFC) has long been used with rodent models to understand the underlying brain mechanisms of psychiatric disorders (e.g. PTSD). Concurrently, MRI techniques, like voxel-based morphometry (VBM), have provided non-invasive ways to study the human brain and associated psychiatric pathology. Within AFC research, several potential circuits that have been proposed, however, not all brain areas have equal evidence and validation. Likewise, VBM has a strong tradition of use in humans, but there is little if any understanding of the underlying changes at the cellular level that account for the signal. Here we attempt to better understand both VBM and AFC by analyzing and correlating small animal MRI imaging and confocal analysis. In particular, Thy1-YFP expressing mice underwent either AFC training or cage handling and then were euthanized two weeks later. The brains were then scanned *ex vivo* with a high resolution T2 imaging protocol in a 9.4 tesla scanner. Following MRI imaging and analysis, the brains were sectioned, stained with Hoechst solution, and imaged with confocal microscopy. The results of the VBM analysis of the brains showed significant increases in grey matter density for the AFC group in the auditory cortex, central and basolateral amygdala, anterior cingulate cortex, and posterior insula. Due to the robust VBM measure and density of Thy1-YFP expressing neurons in the auditory cortex, we focused confocal measures on that brain area. The resulting analysis showed that there was a significant increase in the spine density and spine width with AFC group, but a significant decrease in spine length. Measures of spine density and width were significantly and positively correlated with VBM measures. These results have multiple implications about both AFC and VBM. The VBM results suggest that AFC appears to involve robust structural changes in the auditory cortex, lateral amygdala, anterior cingulate cortex, and insula. The second is that there are concurrent cellular structural changes in the auditory cortex that appear in the form of increased spine density and width. The third is that both the VBM structural measures and the confocal measures of the auditory cortex correlate strongly, suggesting a possible mechanism accounting for VBM signal change.

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Poster

461. Fear and Aversive Memories: Mechanisms

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1F31MH097397

R01MH096764

P51RR000165

Title: Interaction between the cholecystokinin and endogenous cannabinoid systems in cued fear expression and extinction retention

Authors: *M. BOWERS[1], K. J. RESSLER[1,2]
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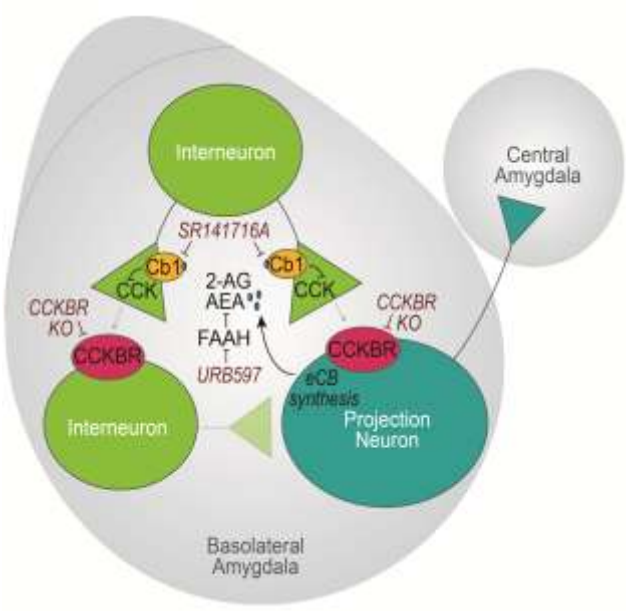
Abstract: Posttraumatic stress disorder (PTSD) is thought to develop, in part, from improper inhibition of fear (Myers and Davis 2007). Accordingly, one of the most effective treatment strategies for PTSD is exposure-based psychotherapy. Extinction of conditioned fear in animal models can be used as an analog of exposure therapy to try to dissect the mechanisms of fear learning. Ideally, neuroscience would inform adjunct therapies that target the neurotransmitter systems involved in extinction processes. Separate studies have implicated the cholecystokinin (CCK) and endocannabinoid systems in fear; however, there is a high degree of anatomical colocalization between the cannabinoid 1 receptor (Cb1) and CCK in the basolateral amygdala (BLA), an area critical for emotion regulation (Josselyn, Frankland, et al. 1995, LeDoux 2000, Marsicano, Wotjak, et al. 2002, McDonald and Mascagni 2001). Although most research has focused on GABA and GABAergic plasticity as the mechanism by which Cb1 mediates fear inhibition, we hypothesize that a functional interaction between Cb1 and CCKBR is critical for fear extinction processes. In this study, systemic pharmacological manipulation of the cannabinoid system modulated cued fear expression in C57BL/6J mice after consolidation of auditory fear conditioning. Knockout of the CCKB receptor (CCKBR), however, had no effect on fear- or anxiety-like behaviors. Nonetheless, administration of a Cb1 antagonist increased freezing behavior during a cued fear expression test in wild-type subjects, but had no effect on freezing behavior in CCKBR knockout littermates. Furthermore, administration of a CCKBR antagonist decreased freezing behavior in Cb1 knockout mice during cued fear expression and extinction retention tests, but had no effect on freezing behavior in wild-type littermates. These findings provide novel evidence that Cb1 contributes to cued fear expression via an interaction with the CCK system. Dysfunctional Cb1-CCKBR interactions might contribute to the etiology of, or result from, fear-related psychiatric disease.



Disclosures: **M. Bowers:** None. **K.J. Ressler:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Title: Behavioral and molecular dissection of Thy-1 expressing neurons in the basolateral amygdala

Authors: ***K. MCCULLOUGH**[1], D. C. CHIO[2], K. J. RESSLER[2]
[2]Psychiatry, [1]Emory Univ., Atlanta, GA

Abstract: The Basolateral Amygdala (BLA) is known to facilitate acquisition and storage of fear memories. Research suggests the presence of distinct circuits within the BLA that play differential roles in the expression of cued fear and fear extinction. Recently, we demonstrated that optogenetic activation of the Thy-1 expressing subpopulation of BLA excitatory neurons during classical conditioning blunts fear acquisition and enhances extinction of fear (Jasnow et al '13). These findings suggest that Thy-1 neurons may represent the Fear $_{OFF}$ population within the BLA previously described physiologically (Herry et al., 2008). Here we further examine the functional role of Thy-1 expressing cells within the BLA as Fear$_{OFF}$ neurons, and we molecularly characterize their mRNA expression profiles. To further investigate the role of activity in Thy-1 neurons in fear extinction, we first utilized a mouse expressing halorhodopsin under the Thy-1 promoter (Thy-1-Halo-YFP). These mice received unilateral ferrule implantation with optical fibers aimed at the BLA, followed by auditory fear conditioning and subsequent extinction training. During extinction the tone was paired with yellow laser stimulation for all mice, inhibiting activity of Thy-1 neurons in halorhodopsin expressing mice. When tested for fear expression to the tone, mice expressing halorhodopsin had significantly more freezing compared to control mice, suggesting that specific inhibition of Thy-1 neurons prevents inhibition of fear behavior. Continuous inhibition of these neurons during fear acquisition resulted in enhanced fear expression tested 24hrs later. To begin to molecularly characterize these cells, Thy-1 expressing neurons from Thy-1 YFP mice were dissociated and isolated by Fluorescence Associated Cell Sorting (FACS). Next generation RNA sequencing was performed on mRNA from sorted cells generating a profile of Thy-1 expressing and Thy-1 negative neurons. We are currently examining these differential RNA profiles in hopes of understanding the molecular signatures of the Fear$_{OFF}$ vs. Fear$_{ON}$ neuronal populations within the BLA. Together these data support the role of Thy-1 neurons as Fear$_{OFF}$ cells and provide a useful profile of their mRNA expression patterns for future targeted pharmacological approaches to manipulating these neuronal populations.

Disclosures: **K. McCullough:** A. Employment/Salary (full or part-time):; Emory University. **D.C. Chio:** A. Employment/Salary (full or part-time):; Emory University. **K.J. Ressler:** A. Employment/Salary (full or part-time):; Emory University.

Poster

461. Fear and Aversive Memories: Mechanisms

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Title: Dexamethasone suppression enhances fear extinction and modulates FKBP5 in a PTSD-like mouse model

Authors: T. SAWAMURA[1], *R. ANDERO GALI[2], T. JOVANOVIC[3], K. RESSLER[1]
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Abstract: Fkbp5 regulates the hypothalamic-pituitary-adrenal (HPA) axis activity during stress response. Also, it is hypothesized that FKBP5 plays a role in the consolidation of both fear and fear extinction memory consolidation. The goal of our study is to understand the mechanisms by which corticosterone and Fkbp5 may mediate these functions in the hippocampus and amygdala in animals previously exposed to immobilization (IMO) to a wooden board, a PTSD-like model (Post-traumatic stress disorder). Six days after the IMO, mice underwent auditory Fear Conditioning (FC) with tone-footshock pairings. Twenty-four hours later, mice received Dexamethasone (Dex, GR receptor agonist), systemically, 4 hours before Fear Extinction, so as to suppress the HPA-axis stress response at the time of fear extinction. Twenty-four hours after extinction, the expression of the extinction memory was evaluated with a Fear Extinction Retention test. We found that Dex enhanced both within-session fear extinction and the retention of fear extinction in this PTSD-like model. Additionally, corticosterone plasma levels and Fkbp5 mRNA in the amygdala were dynamically regulated as a function of both Dex-suppression and fear extinction. Studies are in progress to explain the neural molecular dynamism where corticosterone and Fkbp5 play a role in fear consolidation and extinction within the mouse amygdala.

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Poster

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NIMH (1K01MH085806) (AKS)

Title: A role for HDAC4 in amygdala-dependent fear memory in mice and clinical PTSD in women?

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Abstract: Epigenetic alterations have recently been implicated in the initial consolidation of amygdala-dependent fear memories (reviewed in Maddox et al 2013). While much work has noted the regulation of histone acetylation in fear memory processes, little is understood about the specific role that histone deacetylases (HDACs), which mediate the deacetylation of histone tails, might have in fear memory consolidation. Motivated to understand the epigenetic alterations that may be associated with human clinical PTSD, we performed an epigenome-wide association study (EWAS) in peripheral blood of 410 subjects (57.8% female) obtained from patients in the Grady Trauma Project. We observed that CpG methylation in HDAC4 is associated with PTSD in women (FDR<.05; p<0.01), but not men (p>.05). Further, we determined that HDAC4 CpG methylation appears to influence its expression and have identified several single nucleotide polymorphisms in HDAC4 that associate with PTSD in women. Complementing this work in humans are rodent studies examining the mechanisms of classical fear conditioning, which is a model of the dysregulated fear observed in clinical PTSD. Recent work in rodent models has noted that HDAC4 repression is associated with deficits in spatial learning and hippocampal-dependent contextual fear memory consolidation (Sando et al 2012; Kim et al 2012) suggesting a critical role for HDAC4 in memory processes. However despite this progress, the role of HDAC4 in amygdala-dependent auditory fear memory has not yet been elucidated. Using auditory Pavlovian fear conditioning, we examined the regulation of HDAC4 mRNA in the amygdala 2hr following tone-shock pairings. Using ovariectomized (OVX) female mice with or without estrogen replacement (+E) we revealed higher levels of HDAC4 mRNA in

the amygdala 2h after fear conditioning, compared to OVX-homecage control females. No regulation of HDAC4 mRNA was observed in OVX+E females compared to OVX+E-homecage mice, suggesting a potential link between the hormonal environment and HDAC4 regulation. Additional rodent studies examined the necessity of HDAC4 in the consolidation of auditory fear memories. Thus, these findings suggest that HDAC4 is associated with both fear memory consolidation and estrogen regulation in rodent models. Further, these data implicate an association between clinical PTSD and HDAC4 CpG methylation in females.

Disclosures: S.A. Maddox: None. **B.G. Dias:** None. **K.J. Ressler:** None. **A.K. Smith:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Title: The role of the angiotensin receptor type 1 on corticotropin-releasing factor expressing neurons in auditory fear conditioning

Authors: *R. C. HURT[1,3], O. P. KEIFER, Jr.[2,3], K. J. RESSLER[2,3,4], P. J. MARVAR[5]
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Abstract: Angiotensin II, a component of the renin-angiotensin system, and its receptor, the angiotensin receptor type 1 (AT1), have been studied extensively in the context of blood pressure regulation. However, recently, studies in humans and animal models have suggested a potential role for the AT1 receptor in auditory fear conditioning (Marvar et al., 2013) and post-traumatic stress disorder (PTSD, Khoury et al., 2012). Specifically, angiotensin receptor blockers (ARBs),

such as losartan, have been shown to facilitate extinction of conditioned fear in mice. Concurrently, ARBs were associated with a potential reduction of severity of PTSD symptoms in humans. Importantly, the mechanism of action for these effects is not clear. Therefore, the current study attempts to better understand the underlying neuronal substrates that mediate these effects. We used mice with a conditional knockout of the AT1 receptor gene in corticotropin-releasing factor (CRF)-expressing neurons to investigate the role of angiotensin II in the extinction of conditioned fear. Preliminary results indicate that CRF-neuron AT1 receptor knockout mice acquire fear normally, but express less fear than wild type controls (paralleling results from prior mouse studies with ARBs and human studies with lower expression of PTSD symptoms). Administration of the selective AT1 receptor antagonist losartan reduced this difference by decreasing fear expression in wild type mice, but with no apparent effect in the CRF-neuron AT1 receptor knockout mice. Baseline anxiety and locomotion levels were similar in both groups, as measured by open field and elevated plus maze tests. These results suggest that the action of angiotensin II at the AT1 receptor on CRF-producing neurons is a key component of cued fear consolidation or expression, and that drugs targeting the AT1 receptor may be effective at reducing PSTD symptomology or may serve as adjuncts to exposure therapy for PTSD.

Disclosures: **R.C. Hurt:** None. **O.P. Keifer:** None. **K.J. Ressler:** None. **P.J. Marvar:** None.

Poster

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VA Merit Award 1I01BX001374

Title: Individual differences in behavioral stress responses as a model for PTSD: Exploring cholinergic mechanisms that contribute to fear conditioning responses

Authors: *A. C. SHARKO, K. F. KAIGLER, J. PARRILLA-CARRERO, J. R. FADEL, M. A. WILSON
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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. 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. Our laboratory has demonstrated that outbred Long-Evans rats show individual differences in conditioned anxiety-like behaviors, with significant variability in extinction of freezing behavior following fear conditioning. Approximately 1/3 of each group of rats failed to completely extinguish the conditioned freezing behavior, results that correlate with clinical statistics showing that about 30% of individuals who experience a traumatic event eventually develop PTSD. These same animals demonstrate poor retention of extinction learning 48 hours and 1 week later. To further characterize these individual differences in conditioned behavior, we examined acoustic startle responses in these animals both before and after stress exposure and fear conditioning. We found no significant variability in overall startle response or habituation to acoustic startle at baseline. However, following our fear conditioning protocol, a subset of animals demonstrated significantly increased startle response to an acoustic stimulus. Interestingly, prepulse inhibition was not affected by our fear conditioning protocol and no variability in prepulse inhibition was detected. These behavioral results indicate that this strain may serve as a useful model for characterizing the neurobiological mechanisms that underlie differential sensitivity to traumatic stress. Additional studies examined individual differences in patterns of cholinergic neuronal activation associated with these behavioral patterns, as well as acetylcholine efflux during cue presentation. Enhanced cholinergic signaling promotes anxiety-like behaviors and is implicated in the etiology of stress-related disorders. The basal forebrain cholinergic system may be of particular importance for stress-related disorders as it mediates attention to environmental cues and provides major neuromodulatory input to the basolateral amygdala. Preliminary analysis of Fos expression in cholinergic neurons after extinction trials suggests a relationship between indices of basal forebrain cholinergic activity and individual differences in freezing behaviors seen during extinction trials.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant R01 MH097880

Institutional funds from the Department of Psychiatry at MGH

Title: Predicting the highs and lows of conditioned fear: Mechanisms of estrogen-mediated fear extinction memory consolidation

Authors: *L. Y. MAENG[1], K. K. COVER[4], A. J. LANDAU[4], M. J. WHALEN[2,3], M. R. MILAD[1], K. LEBRON-MILAD[1]
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Abstract: Background: There is a profound sex difference in the prevalence of anxiety- and stress-related psychopathologies such as post-traumatic stress disorder. Evidence implicating a role for gonadal hormones in fear extinction processes is emerging. Specifically, naturally fluctuating levels of estrogen have been found to modulate consolidation of fear extinction memory. In addition, estradiol administration during low estrogen states facilitates memory recall for conditioned fear extinction. In this study, we investigate the molecular mechanisms through which estradiol influences extinction memory within the medial prefrontal cortex (mPFC) and amygdala. Methods: Adult female rats underwent fear conditioning on day 1 and extinction on day 2. Extinction took place in the low-estrogen metestrus phase of the estrous cycle for all animals. Rats received a subcutaneous injection of estradiol (15ug/kg; n=10) or sesame oil vehicle (n=9) 30 minutes before extinction. Brains were collected and processed for immediate early gene c-fos immunohistochemistry and western blot analyses for PI3K (pAkt, pS6) or MAPK (pERK1/2) signaling. Regions of interest were the infralimbic (IL) and prelimbic (PL) areas of the prefrontal cortex and amygdala. Results: Relative to controls, estradiol-treated rats showed a significant increase in c-fos reactivity within the IL ($p<0.05$), but not PL ($p>0.05$), and a trend towards an increase in pERK1/2 expression within the mPFC ($p=0.1$). There were no significant differences in the expression of either pAkt or pS6 within the mPFC. Furthermore, no estradiol-induced differences were observed within the amygdala regarding the expression of pERK1/2, pAkt, and pS6. Conclusions: We provide preliminary data localizing the influence of estradiol administration on extinction-induced neural activity and implicate a molecular pathway that may mediate this effect. Specifically, our findings indicate that estradiol increases extinction-related neural activity within the IL, possibly through MAPK/ERK signaling.

Disclosures: **L.Y. Maeng:** None. **K.K. Cover:** None. **A.J. Landau:** None. **M.J. Whalen:** None. **M.R. Milad:** None. **K. Lebron-Milad:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH061933

NIH Grant R01DA034696

Title: The impact of GIRK2-containing channels on anxiety- and fear-related behavior in mice

Authors: ***N. C. VICTORIA**[1], E. MARRON FERNANDEZ DE VELASCO[1], Z. XIA[1], A. SHNAYDRUK[1], L. KOTECKI[1], M. BENNEYWORTH[2], S. METZGER[2], M. THOMAS[2], K. WICKMAN[1]
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Abstract: Over 40 million adults in the United States suffer from mental illness. Cognitive dysfunction is common in mental illness and is often observed in disorders of anxiety. Regions of the forebrain regulate both cognition and anxiety, including the hippocampus and medial prefrontal cortex (mPFC), whose functioning and interconnectivity become dysregulated in anxiety disorders. Within these regions, G protein-gated inwardly rectifying K^+ (GIRK) channels, which couple to metabotropic receptors and produce neuronal inhibition, are expressed. Notably, global loss of the GIRK2 subunit or GIRK molecular inhibitors decreases anxiety-like behavior and impairs hippocampal-dependent fear memory, respectively. Here, we asked whether loss of GIRK2 in the forebrain, specifically in the hippocampus and mPFC, impairs responses to anxiety- and fear-provoking stimuli in adult male mice. Behavioral, electrophysiological and anatomical assays were used to assess the impact of constitutive, conditional and site-specific viral knockout of GIRK2. In a delay fear conditioning paradigm, freezing was significantly decreased during shock training and 24 hrs later in both context and cue tests for *Girk2–/–* mice, suggesting impaired learning and memory relative to WT controls. Similar to reports in *Girk2–/–*, CaMKII Cre(+):$Girk2^{flox/flox}$ mice that have GIRK2 loss restricted to the forebrain showed increased time in the open arms of the elevated plus maze suggesting anxiolysis relative to Cre(–) mice. CaMKII Cre(+):$Girk2^{flox/flox}$ mice showed impaired fear

learning, but in contrast to *Girk2–/–*, significantly diminished memory in only the context test, suggesting a role for the dorsal hippocampus, potentially the mPFC, but not the amygdala in the observed dysfunction. Somatodendritic recordings from pyramidal neurons in both the dorsal hippocampus and mPFC showed a significant decrease in baclofen induced $GABA_B$-GIRK currents for CaMKII Cre(+):*Girk2*$^{flox/flox}$ mice, indicating reduced neuronal inhibition relative to Cre(–) mice. In addition, immunohistochemistry and quantitative immunoblot confirmed the region-specific loss of GIRK2. Infusion of adeno-associated viral Cre-recombinase/mCherry under control of the human synapsin promoter (AAV-hSyn-mCherry-Cre) into either the dorsal hippocampus or mPFC revealed site-specific impacts on fear learning and memory. Together these data suggest that GIRK2 may play an important role in anxiety and cognition and has implications for pharmacotherapeutic strategies to address mental illness in humans.

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Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: DFG: SFB779/B6

Title: Long-term plasticity and fear learning in adult heterozygous BDNF knockout mice

Authors: S. MEIS[1,2], *V. LESSMANN[1,2], T. ENDRES[1]
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Abstract: The neurotrophin BDNF (brain-derived neurotrophic factor) has been shown to be an important mediator of synaptic plasticity and is crucially involved in learning and memory processes. Several recent studies have demonstrated a critical role of BDNF also in amygdala-dependent cued fear learning. Recently, we could demonstrate that heterozygous BDNF knockout ($BDNF^{+/-}$) mice, exhibit an age-dependent deficit in amygdala-dependent cued fear learning, if they are three months of age or beyond (Endres & Lessmann (2012) Learn. Mem.). In order to pinpoint the underlying synaptic deficits that cause this learning deficit, we now analyzed synaptic plasticity in *in vitro* and *ex vivo* amygdala slice preparations. Since we

previously demonstrated that long-term potentiation (LTP) at thalamic input synapses to the lateral amygdala (LA) is impaired already in one months old $BDNF^{+/-}$ mice (Meis et al. (2012) J.Physiol.), we hypothesized that age-dependent changes of plasticity at the cortico-LA synapses might cause the observed learning deficit. Therefore, we tested LTP at this input structure of the amygdala in 3-4 months old $BDNF^{+/-}$ mice and their wild type (WT) littermates. However, we observed intact LTP in $BDNF^{+/-}$ mice at this synapse as well as at other intra-amygdala synapses, i.e. lateral-basal and basal-central amygdala synapses. To delineate, how fear learning alters synaptic plasticity in the LA, we performed *ex vivo* occlusion experiments. Here we could show, that in fear conditioned WT mice, in contrast to pseudo-conditioned animals, LTP at cortico-LA synapses is occluded 24 h after fear conditioning training, stressing the fear learning relevance of our applied LTP paradigm. Interestingly, LTP at the same synapses was not occluded in fear conditioned $BDNF^{+/-}$ mice. This lack of occlusion 24 h after fear conditioning parallels the fear learning deficit in adult $BDNF^{+/-}$ mice. Currently we are testing occlusion of LTP at cortico-LA synapses 2 h after fear conditioning. First results showed that LTP at this time point is not occluded in adult $BDNF^{+/-}$. This observation is paralleled by an impaired fear memory in these mice at this time point. Interestingly, fear memory was still intact 30 min after fear conditioning, indicating that the acquisition of fear memory is still intact in adult $BDNF^{+/-}$ mice. In conclusion, our results suggest that in adult $BDNF^{+/-}$ mice the acquisition of fear is still intact, indicated by intact short-term fear memory 30 min after fear conditioning. But since we observed neither occlusion of LTP nor intact fear memory 2 and 24 h after fear conditioning, the observed learning deficit seems to result from an unsuccessful early consolidation process.

Disclosures: S. Meis: None. **T. Endres:** None. **V. Lessmann:** None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: USACEHR

Title: Gene expression profiling in a mouse model simulating aspects of PTSD

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Abstract: The social stress (SS) model in C57/BL6 mice reliably elicits PTSD-like behaviors. SS or control (C) mice were housed in a small box (without food or liquid) within a larger cage for 6h, for 5 days or 10 days. SS, but not C, was exposed to an aggressor mouse at random intervals (3x/day). Mice were sacrificed and blood and brain samples taken after 1 day, 10 days or 42 days of rest. Blood samples as well as brain regions associated with fear circuitry were collected from aggressor-exposed (5 or 10 days) or control C57BL/6J mice. The samples were subjected to gene expression profiling, after various periods of rest. Changes in gene expression after each of the 3 periods of rest were profiled using microarray and real time PCR. Published studies of functional and pathway analyses of differentially regulated genes in amygdala, hippocampus, medial prefrontal cortex, ventral striatum and nucleus accumbens of defeated mice have reported an association with startle response, associative learning, long term memory, inflammation, regulation of circadian rhythm, glucocorticoid receptor activity, dendritic branching, and synaptic plasticity. In the present study, transcripts from blood samples demonstrating increased expression following longer rest periods are those known to be associated with T-cell immunity, serotonergic and dopaminergic synaptic processes and axonal guidance. In contrast, transcripts in blood with decreased expression following longer rest periods were those known to be involved in associative learning, opioid signaling and glutamatergic pathways. Changes at the transcriptome level for both blood and brain indicated the pervasive nature of traumatic stress-induced alteration in functions and pathways reported to participate in stress-related phenotypic behavioral disorders and pathologies. After the longer rest period, some of the important functions and pathways tended to return toward control levels, suggesting recovery with time. Key Words: Fear; Stress; Recovery; Mice; PTSD; Gene Expression; Blood; Brain

Disclosures: J.L. Meyerhoff: None.

Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Title: Acquisition of conditioned fear is followed by a transient region-specific increase in unedited kainate receptor RNA

Authors: N. BRANDE-EILAT, Y. GOLUMBIC, *I. GAISLER-SALOMON
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Abstract: Background: Adenosine (A) to inosine (I) RNA editing is a post-transcriptional RNA modification process mediated by specialized enzymes in a site-specific manner. Studies in mammalian brain tissue indicate that the glutamate receptor subunits GRIK1 and GluA2, implicated in learning and memory, undergo A-to-I RNA editing at specific loci. This process leads to a glycine/arginine (Q/R) exchange at the subunit and ultimately results in reduced permeability to Ca2+. We hypothesized that editing rates at Q/R sites of GRIK1 are temporally dynamic and can be modulated by environmental manipulations. Specifically, we posited that learning would be accompanied by a short-term elevation in unedited RNA transcript at GRIK1 and GluA2. Methods: We trained C57/BL6 mice in several fear conditioning (FC) protocols, which are differentially dependent upon intact amygdala and/or hippocampal function. We then examined A-to-I RNA editing levels at the GRIK1 and GluA2 Q/R site in the CA1 and CA3 hippocampal subfields and in the central nucleus of the amygdala (CeA) using a novel TaqMan-based method, at two different time points. Results: unedited RNA levels at the GRIK1 Q/R site were found to be elevated in CA1 and CeA 5 min following hippocampus- and amygdala-dependent trace FC, but not after exposure to unpaired CS and US presentation. Furthermore, unedited RNA levels in CA1 were positively correlated with learning effectiveness in trace FC animals. These effects were found to be transient, as 90 minutes following the behavioral procedure no differences were found between groups. At both time points, no alterations were found in GluA2 editing levels. Conclusions: These findings support the hypothesis that RNA editing is a temporally dynamic process, specifically mediated by behavioral conditioning. In light of the pre-established evidence that decreased editing enables increased Ca2+ influx, the findings suggest that RNA editing at specific loci plays a role in experience-dependent synaptic plasticity.

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Poster

461. Fear and Aversive Memories: Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: IBS

Title: Variability in empathic fear among different inbred mouse strains

Authors: *S. KEUM, A. KIM, K. KIM, H.-S. SHIN
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Abstract: Empathy is an important emotional process that involves the ability to recognize and share emotions with others. We have previously developed a simple behavioral assay to measure fear empathy in mice. In observational fear learning (OFL), mice learn fear without receiving direct adverse stimuli when they observe a conspecific demonstrator receiving repetitive foot shocks. Although we have previously identified that the Cav1.2 calcium channel (Cacna1c) in anterior cingulate cortex (ACC) plays an important role in modulation of OFL, genetic factors regulating empathy are largely unknown. By comparing 10 commonly used inbred mouse strains, we found that fear empathy was highly variable and strain dependent. Four strains- C57BL/6J, C57BL/6N, BTBR T+/J, and 129S4/SvJae- showed significant levels of freezing in OFL, while BALB/cByJ, AKR/J, C3H/HeJ, NOD/ShiLtJ/, DBA/2J, and CBA/J exhibited impaired empathy. To further characterize the differential empathic response between these strains, we conducted a set of behavioral task to examine fear conditioning, anxiety, locomotor activity, and sociability. Most strains that exhibited impaired empathy also showed low levels of freezing in a conventional fear conditioning task. However, despite similar levels of freezing to that of C57BL/6J strain in fear conditioning, BALB/cByJ and AKR/J mice exhibited impaired empathy in OFL task. Anxiety, locomotor activity, or sociability were not correlated with the differential fear empathy between the 10 strains. Taken together, these data strongly suggest that there is a naturally occurring genetic determinant(s) modulating empathy in mice. The identification of causal genes may uncover novel genetic pathways and underlying neural circuits that modulate empathy and, ultimately, provide new targets for therapeutic intervention in human mental disorders.

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Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: NIMH Grant MH 58755

Title: Prospective, concurrent, and retrospective evaluation of outcomes in rodent orbitofrontal cortex

Authors: *Y. JO, S. J. Y. MIZUMORI
Univ. of Washington, Seattle, WA

Abstract: It has been debated whether the orbitofrontal cortex (OFC) encodes subjective reward values or represents the information about outcomes evaluated in a specific situation or state of the task. To address this issue, we recorded single-units from the OFC while 4 rats performed both a delay discounting task and a reward discrimination task on a T-maze. In the former task, the animals were required to choose between a sooner small (SS) reward and a later large (LL) reward. Three different delays to LL reward (10, 20, and 40 s) were used in separate blocks of trials, whereas the delay to SS reward remained constant at 3 s. In the second task, the rats had to discriminate two locations associated with small and large rewards without any delay. We found that different groups of OFC cells exhibited excited responses to one of various salient events and periods in the tasks, such as delay onset, delay period, delay termination, reward, or post-reward period after reward consumption. Individual neurons within these groups exhibited stronger responses to the salient event when a particular delayed reward was chosen compared to the other reward conditions. However, the population activity within each group of OFC cells reflected the relative values of differently delayed rewards evaluated at the salient event. For example, the population responses of delay onset cells were greater at the time of delay onset after rats selected a more desirable reward option (10 s-delayed LL reward) rather than the alternative (3 s-delayed SS reward). Additionally the population activity of OFC cells responding at the time of delay termination, reward receipt, or post-reward was modulated by the amount of reward regardless of delay lengths preceding it. When the same OFC cells were tested in the reward discrimination task, individual cells that responded prior to obtaining reward (i.e., delay onset, delay period, or delay termination) became non-responsive or developed a directional response to one goal arm irrespective of reward size, presumably because rewards were accessible without a delay. By contrast, the OFC cells responding at the time of reward or after consuming reward consistently signaled encountered reward values. Together, these results indicated that individual OFC cells represent information about specific reward conditions in different states of the behavioral tasks, while their population responses signal subjective values of all reward conditions evaluated in the corresponding states. These neural correlates highlight the essential roles of the OFC in decision making.

Disclosures: Y. Jo: None. **S.J.Y. Mizumori:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: Italian Ministry of Health "Ricerca Corrente 2013".

Title: Prefrontal cortical Norepinephrine delays extinction of amphetamine-induced conditioned place preference

Authors: E. LATAGLIATA[1], P. SACCOCCIO[2], C. MILIA[2], *S. PUGLISI-ALLEGRA[2,1]
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Abstract: Environmental stimuli or discrete cues paired with addictive drugs are considered major factors able to trigger intense drug desire that can lead, humans and animals, toward drug-seeking behavior. Recent evidence indicates medial prefrontal cortex (mPFC) as critical for expression and extinction of conditioned behavior in appetitive and aversive domains. Studies conducted in our lab have demonstrated that selective depletion of norepinephrine (NE) in the mpFC interferes with acquisition of conditioned place preference (CPP) based on different addictive drugs or natural rewards. We assessed whether and how prefrontal NE affects the persistence of drug-associated cue memories. First, we investigated the effects of selective NE depletion in mpFC (including prelimbic and infralimbic areas) on retrieval, extinction of the acquired CPP to amphetamine in C57BL/6J mice in a spontaneous extinction paradigm (subsequent daily testing sessions). Second, we assessed the effects of alpha-1 adrenergic receptor antagonist (Prazosin) infusion in mpFC on extinction of the acquired CPP to amphetamine. NE depleted mice extinguished place preference after eight extinction sessions, while, Sham group maintained the preference until the fourteenth day from the beginning of extinction procedure. In Prazosin treated mice the preference is blocked from the first day of extinction, while in Sham group the preference persists for further thirteen days. These results indicate that prefrontal cortical NE contributes to delay extinction of memories associated with drugs, and that alpha-1 receptors in mpFC play a critical role in this process.

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Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R00 DA024719

Title: Prefrontal cortical deletion of DNA methyltransferases Dnmt1 and Dnmt3a induces anhedonia

Authors: *J. V. JOY-GABA[1], D. WARTHEN[1], B. NEWMYER[1], P. LAMBETH[1], J. A. JOY-GABA[2], R. GAYKEMA[1], M. SCOTT[1]
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Abstract: DNA methylation is an epigenetic mechanism associated with the repression of gene expression; mediated by the DNA methyltransferases (Dnmt) which catalyze the addition of a methyl group to the 5' position of cytosine in DNA. Expression of both Dnmt1 and Dnmt3a isoforms within post-mitotic neurons of the central nervous system has been shown to be important in modulating the response to drugs of abuse and other stimuli that produce changes in neuronal plasticity. Interestingly, Dnmt1 and Dnmt3a expression has been shown to be dynamically regulated in the medial prefrontal cortex (mPFC), suggesting that these enzymes may also be involved in the modulation of mPFC dependent behavior. In a test of this hypothesis, we examined the requirement for Dnmt mediated methylation in regulating PFC-dependent reward behavior. We generated mice with a PFC-specific deletion of Dnmt1 and Dnmt3a, and assayed for changes in several PFC-dependent behaviors. Although no differences were observed in operant responding for food reward, daily food consumption, novel environment preference, open field, or elevated plus maze, mice lacking Dnmt expression in the PFC consumed less in a binge-like feeding assay when presented with either high-fat chow or high starch chow compared to regular chow. Furthermore, these mice displayed a reduction in social interaction and a reduced preference for sucrose solutions. Taken together, our data are the first to show the requirement for a specific epigenetic process in the regulation of both feeding behavior and affect.

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Poster

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Location: Halls A-C

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Program#/Poster#: 462.04/TT40

Topic: F.02. Animal Cognition and Behavior

Support: IAF311009

Title: Population decoding in the frontal eye fields and prefrontal cortex during a visual working memory task

Authors: *A. PARTHASARATHY[1,2], R. HERIKSTAD[1,3], J. BONG[1,3], C. LIBEDINSKY[4], S.-C. YEN[1,3]
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Abstract: Background: The frontal eye fields (FEF) and the dorsolateral prefrontal cortex (dl-PFC) are two prefrontal regions that share direct reciprocal anatomical connections, and are thought to be involved in target selection, planning and execution of the saccade. dl-PFC is also known to exhibit responses that indicate the maintenance of information in working memory. Despite the clear integration of these two regions in visuo-motor processing, no studies to date have analyzed the activity of both FEF and DLPFC simultaneously to understand the dynamics of the interaction between them. Decoding algorithms were used to understand the information in the neural responses. Methods: Single neurons were recorded in an awake-behaving monkey (Macaca fusicularis) using chronically implanted electrodes while the animal performed a visual fixation task. 32 electrodes were implanted in FEF and 96 electrodes in dl-PFC. The monkey was trained to perform a memory guided saccade task. The task also included a distracter that the monkey was expected to ignore. Eye movements were monitored using an infrared eye tracker system. Correct and incorrect trials were analyzed in this study. We decoded the location of the target using the receptive fields of each of the simultaneously recorded single units, weighted by the corresponding spike counts at various time points during the trial. Results: Our results showed that, in single trials, the information encoded by the activity of a population of neurons was much higher than that of single cells. We used the performance of a linear discriminate analysis as a measure to indicate the information encoded by the neural activity. The classifier could predict the location of the target in 83% of trials using the population activity and 50% of the trials using single cell activity The decoder based on the population activity of neurons performs correctly in 80% of the trials during the target presentation and during the delay period. However, this performance drops to about 55% upon the introduction of a distracter. In addition, we found evidence that on 56% of the error trials, the decoder was not able to decode the target position correctly during the target presentation, suggesting that the animal failed to encode the

stimulus location correctly. In the remaining 44% of the error trials, the decoder was able to decode the target position correctly, suggesting that the animal encoded the stimulus location correctly, but in 70% of these trials, the decoder was unable to decode the target location correctly during the delay, suggesting that the memory of the target location failed to be maintained successfully.

Disclosures: A. Parthasarathy: None. **R. Herikstad:** None. **J. Bong:** None. **C. Libedinsky:** None. **S. Yen:** None.

Poster

462. Prefrontal Cortex II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 462.05/TT41

Topic: F.02. Animal Cognition and Behavior

Title: Bayesian analysis provides additional insight into the set shifting deficits in orbital frontal cortex lesioned rats

Authors: *A. WHYTE, J. WANG, E. A. CHASE, D. TAIT, V. J. BROWN, E. BOWMAN
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Abstract: The attentional set-shifting task measures behavioural flexibility and, because it is translatable between humans and rats, it is used as a measure of fronto-striatal network function in rodent models of executive dysfunction. The subject learns a series of two-choice discriminations to a criterion level of performance, which is typically 6-correct-in-a-row ($p=0.015$). This approach is a traditional 'null vs. alternative hypothesis' framework, with 'random-guess' implied as the null hypothesis. However, there are other reward-irrelevant strategies that need to be rejected. A Bayesian approach calculates the a posteriori probability of any given strategy being used from the entire response history, as opposed to solely considering on the correctness of the responses in the 6-correct-in-a-row criterion. We identified nine potential strategies that a rat might use in the task: five spatial (right; left; alternate; win-shift; win-stay) and four perceptual (choice of each exemplar). All probabilities were initially equivalent, but with each choice, the Bayesian probabilities were updated. The criterion for learning was when the probability of the correct exemplar was .95. Using this method, we reanalysed archival data from Lister Hooded (Charles River) rats with ibotenic acid lesions to the orbitofrontal cortex or sham surgery. It is possible for the 6-correct-in-a-row criterion to be met without the Bayesian criterion, and vice versa. Trials after Bayesian criterion were classified as

overtraining and trials before as undertraining. For both groups, the number of overtraining trials during the initial stages of the test were positively correlated with trials to criterion in the ED-shift. We previously reported that these lesioned rats are impaired in set formation, and when set was formed, the ED-shift was impaired. Consistent with this, there was a negative correlation between the number of overtraining trials in the ID stages and the trials to (6-in-a-row) criterion in the ED. The lesioned rats were also more likely to have undertrained stages. We conclude that this Bayesian analysis provides additional information about why the rats do not form set.

Disclosures: **A. Whyte:** None. **J. Wang:** None. **E.A. Chase:** None. **D. Tait:** None. **V.J. Brown:** None. **E. Bowman:** None.

Poster

462. Prefrontal Cortex II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 462.06/TT42

Topic: F.02. Animal Cognition and Behavior

Title: Testing computational models of anterior cingulate cortex with monkey single units

Authors: A. JAHN[1], C. STRAIT[3], *J. W. BROWN[2], B. HAYDEN[3]
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Abstract: A number of theories have been proposed to account for the role of anterior cingulate cortex (ACC) and the broader medial prefrontal cortex (mPFC) in cognition. The recent Prediction of Response Outcome computational model (PRO model; Alexander & Brown, 2011) describes the mPFC as performing two theoretically distinct functions: learning to predict the various possible outcomes of actions, and then evaluating those predictions against the actual outcomes to yield a prediction error. Here we use single-cell recordings in primates to test the PRO model predictions and compare them against other established models of mPFC function, including the Reinforcement Learning model (Holroyd & Coles, 2002). We isolated prediction and prediction error signals, and we separated them from preceding visual and motor signals. Our results show cells within the dorsal ACC (dACC) that signal imminent predicted outcomes. These cells have activity that ramps up toward a predicted outcome. We also found cells with qualitatively different neural firing for surprising outcomes as opposed to non-surprising outcomes. Critically, we find certain cells more sensitive to worse-than-expected outcomes, some cells more sensitive to better-than-expected outcomes, and some cells responsive to both

worse-than-expected and better-than-expected outcomes. These findings are consistent with distinct prediction and evaluation signals as posited by the PRO model and provide new perspective on a large set of known effects within ACC.

Disclosures: **A. Jahn:** None. **J.W. Brown:** None. **C. Strait:** None. **B. Hayden:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.02. Animal Cognition and Behavior

Support: Staglin Family and International Mental Health Research Organization

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NIDA (R01DA035913 to KB),

NIGMS (Medical Scientist Training Grant GM07618 to AL)

NIH Office of the Director (DP2MH100011)

Title: Bidirectional modulation of behavioral flexibility by tonic and phasic stimulation of ventral tegmental inputs to the prefrontal cortex

Authors: *I. T. ELLWOOD, T. PATEL, A. T. LEE, A. T. LIPTAK, K. J. BENDER, V. S. SOHAL
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Abstract: An important function of the prefrontal cortex (PFC) is adapting behavior to changes in the external world. Input to the PFC from the ventral tegmental area (VTA) is hypothesized to play a critical role in this process, by linking signals from the environment with appropriate changes in PFC function. Specifically, phasic or tonic modes of VTA neuron firing are believed to signal unexpected or expected events in the external world, respectively. These two patterns of VTA neuron output may then elicit appropriate changes in PFC-dependent behavior by generating different levels of prefrontal dopamine, which activate different subtypes of dopamine receptors. However, at present, little is known about whether these two modes of VTA output can in fact elicit distinct changes in PFC-dependent behavior as hypothesized. Here, we show

that optogenetic stimulation of dopaminergic projections from the VTA to the PFC in TH-Cre mice influences whether mice change or maintain a behavioral strategy in a manner that depends on the pattern of stimulation. Under tonic stimulation, mice were highly perseverative, following an outdated strategy even when the rules of a task changed. In contrast, stimulating VTA to PFC projections using phasic bursts induced an apparently random strategy, in which mice neither adapted to the new rules of a task, nor continued following old strategies. Finally, we give evidence that these effects are potentially driven by a combination of dopaminergic modulation and direct glutamatergic excitation.

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Poster

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Support: NSF DMS-1225647

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2R01MH065252-06

Title: Beta spike-field coherence in a flexible categorization task: a single prefrontal neuron ensemble for multiple categorization schemes

Authors: ***D. A. STANLEY**[1,2], K. G. BUCHANAN[3], J. E. ROY[4], E. K. MILLER[4], N. J. KOPELL[2]
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Abstract: Flexible categorization involves classifying an object according to different categorization schemes, depending on context. For example, a lion can be categorized as a potential predator or as a type of feline. In this study, we examine beta (19-40 Hz) spike-field coherence (SFC) during flexible categorization. First, we show that a single ensemble of neurons exhibits beta SFC for two distinct categorization schemes. Second, the SFC of an individual

neuron increases with its ability to multiplex both categorizations. Two Macaca mulatta monkeys (8-10 kg) were presented with computer generated animal images and were cued to perform one of two categorizations: cat vs. dog or fat vs. thin (Roy et al., 2013). We evaluated the ability of neurons to perform a given categorization (i.e. cat vs. dog) by testing for significant differences in firing rate across images in each category (t test, Bonferroni corrected). We then grouped neurons based on their ability to perform the categorization scheme indicated by the current cue. For example, some neurons (Gr 1) categorized correctly for only one cue, whereas others (Gr 2) actually switched between performing cat-dog and fat-thin categorizations, effectively performing the correct categorization for both cues. A third group were insensitive to image category (Gr 0). We analyzed SFC and, also field-field coherence (FFC) between electrodes, using standard coherence measures in the Chronux MATLAB package (Bokil H et al., 2010). We found that the beta SFC of a neuron increased with its relevance to the overall task. Specifically, the group of neurons that could multiplex both categorizations (Gr 2) had higher beta SFC than those performing only one categorization (Gr 1), which in turn were higher than the remainder of the population (Gr 0) (ANOVA, p=3.8e-11). Furthermore, for the majority of neurons (97%), SFC was not sensitive to the current categorization scheme. For example, a typical neuron would show the same level of SFC when cued for both cat vs. dog and fat vs. thin categorizations. These findings suggest that the same neural ensemble is coherent during both categorization schemes, and that the neurons in this ensemble are most relevant to the overall flexible categorization task.

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Poster

462. Prefrontal Cortex II

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Program#/Poster#: 462.09/TT45

Topic: F.03. Motivation and Emotion

Support: Intramural Research Program of NIDA/NIH

Title: Rewarding effects of optogenetic stimulation of the medial prefrontal cortex and adjacent regions in mice

Authors: C. YANG, *S. IKEMOTO
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Abstract: The medial network (MN) of the frontal cortex has been implicated in affective disorders such as depression. The MN includes the prelimbic (PrL), intralimbic (IL), and medial orbital (MO) areas. In addition, the MN may include, in the rodent brain, the tenia tecta (TT), which is strongly connected with the PrL, IL and MO, and, just like other MN structures, receives afferents from the thalamic nuclei and may project to the ventral striatum and lateral hypothalamic area. Little is known about the roles of MN glutamatergic and GABAergic neurons in affective process. We sought to understand them using optogenetic and behavioral procedures in mice. Here we summarize a preliminary set of data involving non-selective stimulation of MN regions and suggesting MN's role in reward. Male C57BL/6J mice received adeno-associated viral vectors encoding channelrhodopsin-2 into the PrL (n = 5), IL (n = 5), MO (n = 4), dorsal TT (n = 5) or ventral TT (n = 5), resulting in expression of the opsin in neurons of respective regions. These mice were trained to press on a lever for a train of photostimulation consisting of 8 pulses (3 ms duration) delivered at the 25-Hz frequency, which phasically excited neurons. Mice quickly learned to leverpress over 5 daily sessions (30 min per session). In the 1st 30-min session, mice pressed lever 597, 635, 656, 298, and 118 times for the photostimulation of PrL, IL, MO, dorsal TT and ventral TT, respectively. In the following training sessions, the number of lever presses gradually increased, attaining mean lever presses of 1314, 1196, 1279, 457, and 228 in the 5th 30-min session. In addition, they rapidly reduced leverpressing during extinction and quickly re-acquired it when photostimulation was reinstalled. We then examined effects of stimulation frequencies at 6.25, 12.5, 25, 50 and 100 Hz. While mice self-stimulated with all five frequencies, differences between the five frequencies were significant, and the 50-Hz frequency was most effective. In conclusion, phasic excitation of MN neurons induces reward.

Disclosures: **C. Yang:** None. **S. Ikemoto:** None.

Poster

462. Prefrontal Cortex II

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Program#/Poster#: 462.10/TT46

Topic: F.02. Animal Cognition and Behavior

Support: The Education Committee of the Beijing City, KM201410028019

Title: The different predicting roles of the anterior cingutate cortex and the orbital frontal cortex in risk-taking tasks in rats

Authors: *P. YU, R. XU, H. LI, A. JI
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Abstract: The anterior cingutate cortex (ACC) and the orbital frontal cortex (OFC) are two areas that have been known to participate in the decision making process. Previous studies have shown that ACC might participate in the formation of strategies and the execution of actions, the OFC is regarded as an important area for the learning and updating of strategies. The ACC is more sensitive to the value of reward and responsible for action-oriented behaviors, while the OFC mainly receives information from multiple sensory input systems, and is responsible for sensation-oriented behaviors. In risk-taking tasks, however, the difference remains unclear between the ACC and OFC on encoding and predicting the reward and risk information. Our study attempts to address to this issue. Unit activities in the period of prediction were observed in probability-discount tasks by multichannel single-unit recording in rats. Big rewards were set up at four probabilities: 100%, 50%, 25% and 12.5% with the contrast small reward at 100% probability. Two classes of anticipation neurons (excitatory and inhibitory) were observed in the ACC. For excitatory neurons, there was no difference on burst frequency whether rats chose to take the risky or the safe options, but significant difference among probabilities. The results showed that this class of neuron was engaged in predicting the incoming risk and directing behaviors based on the prediction. On the contrary, the ACC inhibitory neurons fired more vibrantly when animals chose to take the safe options. The finding suggests that this class of neurons could only predict the value of the behavior but not the risk. Two classes of anticipation neurons (excitatory and inhibitory) were recorded in the OFC as well. Different from the ACC, these neurons have statistically the same firing rate in risky and safe options among four different probability blocks. The results suggest that the OFC could not predict the value of a reward, nor the risk, especially after the learning when the OFC might not majorly participate in the process. The conclusion is that the ACC is responsible for predicting the risk of the behaviors and the value of the reward, so as to command the motor system to take actions accordingly. The OFC, on the other hand, does not predict the incoming risk after the rats learn the rules of the task.

Disclosures: **P. Yu:** None. **R. Xu:** None. **H. Li:** None. **A. Ji:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.02. Animal Cognition and Behavior

Support: NIAAA R01AA020394-01 to BMW

Title: Kappa opioid-receptors in the medial prefrontal cortex: Endogenous regulators of cognitive control

Authors: *S. SIROHI, B. M. WALKER
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Abstract: Impulsivity is more prevalent in younger population and declines with maturity. In the present study, we found that kappa-opioid receptor (KOR) stimulation significantly increased impulsive responding in young rats compared to mature rats when tested using the Stop-Signal Reaction Time Task (SSRT). Considering that a substrate of SSRT performance is the medial prefrontal cortex (mPFC), we assessed KOR function in the mPFC using an endogenous ligand for the KOR. Dynorphin (DYN) A-stimulated GTPyS coupling was significantly higher in the mPFC of young rats compared to mature rats. Such data suggest that mPFC KORs may play a role in the observed age-dependent decline in impulsive-like behavior. Deficits in impulse and cognitive control are symptoms of alcohol dependence and we have previously shown alcohol dependence-induced dysregulation of KOR function in other brain regions. Therefore, we next assessed functional changes in mPFC KOR signaling induced by alcohol dependence using the DYN A-stimulated GTPγS assay. The results showed that KOR signaling was significantly elevated in the mPFC of alcohol-dependent (vapor-exposed) compared to the non-dependent (air-exposed) animals. These data implicate mPFC KORs in age-dependent alterations in impulsivity, as well as the dysregulation of those behaviors observed in alcohol dependence. Interestingly, the functional activity of mPFC KORs of mature non-dependent animals was profoundly attenuated, whereas KOR function was increased in mature alcohol-dependent animals. This demonstrated a 'normal' maturational decline in KOR function, which is reversed by chronic alcohol exposure and suggests that KORs can exhibit a quiescent profile under certain conditions. Lastly, given that a link between high impulsivity and impaired working memory has been shown and both impulsivity and working memory deficits are symptoms of alcohol-dependence, we next evaluated the effects of KOR agonist infusions in the mPFC to alter performance in a measure of working memory, the Delayed Non-matching to Sample Task. Intra-mPFC infusions of the KOR agonist U50488 induced working memory deficits, an effect that was completely rescued by the pretreatment with the KOR antagonist nor-binaltorphimine which supports the hypothesis that dysregulation of the mPFC DYN/KOR system could underlie impairments in impulse control and working memory observed in alcohol-dependent populations. Of critical importance, a novel therapeutic indication was identified for the use of KOR antagonists to treat impulse control disorders and working memory deficits that are symptomatic of alcohol dependence and other neuropsychiatric disorders.

Disclosures: S. Sirohi: None. **B.M. Walker:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: funds from the State of California through UCSF

Title: Orbitofrontal cortex mediates cue-evoked inhibition of basolateral amygdala neurons during appetitive Pavlovian conditioning

Authors: *K. R. VITALE, P. H. JANAK
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Abstract: Pavlovian conditioning is mediated in part by strengthening of excitatory synapses onto basolateral amygdala (BLA) neurons that carry sensory information about the conditioned cue (Tye et al., 2008; Clem and Huganir, 2010). The resulting activation of BLA neurons during cue presentation is thought to underlie conditioned behavioral and physiological responding to the cue. In general agreement with this model, single unit recording studies have found that a subset of BLA neurons is excited by a conditioned cue after either appetitive or aversive Pavlovian conditioning (e.g. Shabel and Janak, 2009). To further explore how the amygdala contributes to this learning process, we recorded single-unit activity in the BLA of male rats as they underwent appetitive Pavlovian conditioning. Training consisted of repeated presentations of a 30s auditory stimulus (CS+) paired with sucrose solution delivered at a variable latency and an equal number of presentations of a different sound (CS-) paired with nothing. Interestingly, we found that nearly half of recorded pyramidal neurons were inhibited by the CS+, whereas <10% were excited by this cue. In addition, BLA inhibition was highly correlated with conditioned behavior in all phases of training: acquisition, expression, and extinction. Because the BLA is a heterogeneous population of neurons that contributes to both aversive and appetitive behavior, one possible role for cue-evoked inhibition in BLA is to suppress competing behaviors and thereby facilitate decision making during well-trained Pavlovian responding. To investigate this possibility, we examined the effect of pharmacological inactivation of the orbitofrontal cortex (OFC), an area involved in decision making, on BLA inhibitory signaling of the CS+. We found that cue-evoked inhibition of BLA neurons and conditioned behavior are impaired after OFC inactivation, suggesting that OFC mediates this signal and also contributes to Pavlovian conditioned responding. Although OFC projections to BLA are glutamatergic, OFC could promote feed-forward inhibition of BLA pyramidal neurons via activation of local interneurons or lateral paracapsular neurons. This research is the first to suggest a role for BLA

inhibition or an OFC-BLA circuit in Pavlovian conditioning, and expands our understanding of how cortico-amygdalar interactions contribute to motivated behavior.

Disclosures: K.R. Vitale: None. **P.H. Janak:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.02. Animal Cognition and Behavior

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Royer and Clayton Family

Title: Rule training enhances structural plasticity of long range projecting orbitofrontal cortex axons

Authors: C. M. JOHNSON[1], H. PECKLER[1], *L. E. WILBRECHT[2]
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Abstract: The frontal cortex is involved in learning abstract rules to guide complex goal directed behavior, though we know very little about how these rules are stored at the synaptic level. The orbitofrontal cortex (OFC) and the dorsomedial frontal cortex (dmPFC) have both been implicated in rule learning, and likely integrate information about sensory cues and expected outcomes with motor action plans. Here, we used *in vivo* two-photon imaging to investigate the dynamics of axonal boutons that connect the OFC with the dmPFC in mice trained on an odor discrimination and reversal task previously shown to rely on the integrity of these regions in rodents (Kim and Ragozzino, 2005; Johnson and Wilbrecht, 2011). We found enhanced turnover of boutons as soon as two hours following rule training compared to untrained mice. Bouton turnover significantly correlated with performance on the odor discrimination (R=0.67). Following odor discrimination learning, trained mice also had significantly higher gains of boutons, in particular new boutons that persisted through the end of training. In contrast, reversal

learning was followed by loss of previously stable boutons, which significantly correlated with reversal performance (R=0.69). Bouton dynamics during a baseline period before training did not correlate with later task performance. A separate group of mice were allowed to explore the behavior arena and odor pots, but without food rewards or rule training. This enrichment group had indistinguishable bouton dynamics compared to standard housed mice. Together, these results indicate that rule training, but not novelty or reward alone, results in lasting modifications to a frontal cortex circuit.

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Poster

462. Prefrontal Cortex II

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Title: Adolescent female mice exhibiting activity-based anorexia express elevated inhibitory input onto prefrontal cortical layer V pyramidal cells with axon terminal restructuring that is distinct for ventral versus dorsal subregions

Authors: *Y.-W. CHEN, G. S. WABLE, T. G. CHOWDHURY, C. J. AOKI
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Abstract: Anorexia nervosa (AN) is a psychiatric disorder with one of the highest mortality rates, but no accepted pharmacological treatment. Physical hyperactivity plays a central role in the pathogenesis and progression of the disorder. Functional brain imaging (fMRI) studies of AN patients have identified altered prefrontal cortex (PFC) activations. Activity-based anorexia (ABA), a rodent model of AN, which combines food restriction (FR) and wheel running, results in severe weight loss and ultimately death unless the animal is removed from the ABA-inducing environment. Previous findings showed that a positive correlation between metabolism in the anterior cingulate cortex and body weight loss in ABA rats. The aim of this study was to investigate the effect of two ABAs, spaced a week apart, on the inhibitory input onto the prefrontal cortical pyramidal neurons. In order to assess the extent of inhibitory inputs, we quantified the proportion of the plasma membrane of layer V pyramidal neurons contacted by axons immunoreactive for GAD in Cg1, PrL, and MO regions of the PFC through electron microscopic immunocytochemistry. We show that (1) The degree of the hyperactivity after the onset of FR was variable across individuals. (2) In all three regions of PFC, the cell body profiles of the pyramidal neurons in ABA animals were ~43% more extensively contacted by GAD-terminals than controls ($p < 0.05$). (3) In Cg1, there was no correlation between running activity prior to FR and GAD terminal lengths. However, GAD terminal lengths were negatively correlated with the wheel activity during the first ABA ($R = -0.7568$, $p < 0.05$), meaning that the animals exhibiting the greatest increase in wheel activity were the ones that exhibited the lowest increase of GAD terminal lengths. The negative correlation was even stronger during the second ABA exposure ($R = -0.8748$, $p < 0.01$). The negative correlation between GAD terminal length and total running activity was also strong ($R = -0.8116$, $p < 0.05$). These strong correlations were specific for the Cg1, in that the PrL and MO did not exhibit this correlation. (4) A positive correlation was found between GAD terminal lengths and food anticipatory activity (wheel running during the 6 hour bin that immediately precedes feeding) of the first ABA in the PrL ($R = 0.5559$, $p = 0.05$) and marginally also in the MO ($R = 0.4814$, $p = 0.08$), meaning that the animals exhibiting the greatest increase in wheel activity during the FAA period were the ones that exhibited the greatest increase of GAD terminal lengths. These findings highlight a dorsal/ ventral distinction of ABA-induced inhibitory alteration in the murine PFC.

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Poster

462. Prefrontal Cortex II

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 462.15/TT51

Topic: F.02. Animal Cognition and Behavior

Title: The effect of prefrontal and/or hippocampal lesions on performance of cognitive shifts in a T-maze

Authors: *H. MALA, L. G. ANDERSEN, R. F. CHRISTENSEN, A. FELBINGER, J. HAGSTRØM, D. MEDER, H. PEARCE, J. MOGENSEN
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Abstract: Within one experiment and one T-maze, we examined the consequences of i) bilateral lesions of the anteromedial prefrontal cortex (PFC), ii) bilateral transections of the fimbria-fornix (FF), or iii) combined lesions of both PFC and FF (COMB) on rats' ability to perform an intradimensional or extradimensional shift. Postoperatively, the animals were trained to perform a spatial discrimination go-right task. This was followed by 1) a spatial reversal go-left task (intradimensional shift), or 2) a visual pattern discrimination task (extradimensional shift). Regarding the intradimensional shift, the performance of the PFC group was not different from that of the sham operated control animals (Sham). In contrast, animals with FF lesion only or a combined lesion of both structures were impaired on error rate. The COMB group was also impaired compared to the FF group. Regarding acquisition speed, only the COMB group was impaired relative to all other groups. Hence, the hippocampal lesion in isolation affected only the ability to perform a reversal, but not the acquisition speed. Regarding the extradimensional shift, all lesioned groups were impaired relative to the Sham group both regarding the error rate and the acquisition speed. There was, however, no difference in the degree of impairment between the lesioned groups. We conclude that both the PFC and the hippocampus contributed to the mediation of the intra- and extradimensional shifts. During functional recovery of intradimensional shifts, these two structures exhibited a mutual dependency, while the functional recovery of extradimensional shifts was mediated by a substrate outside these two structures.

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Poster

462. Prefrontal Cortex II

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Topic: F.02. Animal Cognition and Behavior

Support: iaf311009

Title: Information transfer between frontal eye fields and dorsolateral prefrontal cortex in macaques during a visual working memory task

Authors: *R. HERIKSTAD[1,2], J. H. BONG[1,2], C. LIBEDINSKY[3], A. PARTHASARATHY[4,2], S.-C. YEN[1,2]
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Abstract: Background: The frontal eye fields (FEF) and the dorsolateral prefrontal cortex (dl-PFC) are two prefrontal regions that share direct reciprocal anatomical connections, and are thought to be involved in saccade generation and the deployment of voluntary visual attention. Both regions contain neurons that respond either to visual stimuli, around the time of eye movements, and/or to spatial locations stored in working memory. While the single-cell behavior is well understood, less is known about the functional connectivity between these two regions. Methods: We recorded populations of neurons in the behaving monkey (Macaca fusicularis) using chronically implanted electrodes (32 electrodes in FEF, 48 in dl-PFC) while the animal was engaged in a delayed memory saccade task with a distractor. We analysed both correct and incorrect trials. For both types of trials, we binned the neural responses aligned to target onset using 20 ms bins. We then computed the transfer entropy as the difference in entropy between one cell's responses conditioned on its own history, and the entropy of the same cell's responses conditioned on both its own history and the history of a second cell. The transfer entropy was deemed significant if it exceeded the 99th percentile of shuffled surrogates for at least 3 consecutive bins. Results: We recorded 130 cells over 4 sessions. The largest number of simultaneously recorded cells was 58. For correct trials, we found 520 pairwise connections with significant transfer entropy during the time period before the distractor presentation. Out of these, 23% (123/520) exhibited significant transfer entropy after the distractor onset in error trials. The majority of connections exhibiting this effect occurred within the dl-PFC (97), while 11 went from FEF to dl-PFC, 12 from dl-PFC to FEF and 3 occurred within FEF. Furthermore, cells that encoded information about the target during correct trials had more outgoing connections compared to non-informative cells during correct trials (4.3±2.1 vs 3.2±2.4, $p = 0.04$). However, during incorrect trials, the number of outgoing connections was not different for the two types of cells (22±7.7 vs 20±7.1, $p=0.14$). Conclusion: Increased connectivity during error trials after distractor onset could indicate that in those trials, the distractor was encoded by a subset of neurons, which then transferred information about the distractor to other cells as though it were a target. Evidence of overall increased, but less specific, connectivity during error trials furthermore suggests an increased level of uncertainty about the target in the network for those trials.

Disclosures: **R. Herikstad:** None. **J.H. Bong:** None. **C. Libedinsky:** None. **A. Parthasarathy:** None. **S. Yen:** None.

Poster

462. Prefrontal Cortex II

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NARSAD

Title: Prefrontal neural dynamics during decision-making process

Authors: ***H.-A. TSENG**, X. HAN
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Abstract: In daily life, animals constantly make decisions, and being able to make proper decision based on both the sensory information and the prior experience is crucial for their survival. Many studies have suggested decision-making involves prefrontal cortex, and therefore it has become a great interest to understand the prefrontal neural dynamics during the decision-making process. Here, we examined the neural activity in mouse prefrontal cortex when mice were performing an auditory discrimination task. During the task, mice initiated each trial by triggering the start-spot at the one side of the behavior box, and one of three sounds (10k Hz sine wave, 25 clicks/second, and 100 clicks/second) would present throughout the trial. Mice were trained to distinguish the sound and moved to the one of the three reward-spots at the other side within a limited time window (10 second). When choosing the correct reward-spot, mice received a drop of water as reward. Choosing an incorrect reward-spot or failing to choose within

the time window resulted in a timeout for 5 second. A well-trained mouse can finish each trial in 1-2 seconds with correct rate over 80%. During the whole process, we use tetrode device to record the spike activity and the local field potential (LFP) at four different locations in the prefrontal area. Our results show that during the auditory discrimination task, distinct groups of prefrontal neuron increased their firing rate at the different stages of the decision-making process, indicating that neural activity could code for the trial progress. Besides firing rate, we also examined the LFP spectrogram during the task and found that the strength of the oscillation at beta frequency is associated with the stages of the task. Upon the start of each trial, as the mice received the sound, the beta power first reduced and then rebounded after ~0.5 second. The drop of beta power occurred again at the end of the trial. Therefore, the decrease of beta power is linked to the sensory inputs during the task, such as the sound and the reward. Overall, our results suggest that neural activity in the prefrontal area exhibits a progress-dependent dynamic during the decision-making process.

Disclosures: H. Tseng: None. **X. Han:** None.

Poster

462. Prefrontal Cortex II

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: DP130103965

Title: Medial orbitofrontal cortex mediates use of outcome representations when outcomes are unobservable

Authors: *L. A. BRADFIELD[1], M. VAN HOLSTEIN[2], B. W. BALLEINE[1]
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Abstract: The role of the medial orbitofrontal cortex (mOFC) in instrumental conditioning was assessed in three separate studies using rats. First pre-training mOFC lesions were found to impair specific Pavlovian-instrumental transfer (sPIT) and outcome devaluation, but spared contingency degradation and outcome-induced reinstatement. In the second study the adeno virus AAV-hSyn-HA-hM4D(Gi)-IRES-mCitrine was stereotaxically injected into the mOFC to promote the viral-mediated expression of hM4D: Gi/o coupled muscarinic designer receptors

exclusively activated by designer drugs (DREADDs). Rats were then trained and tested for sPIT and outcome devaluation after receiving interperitoneal injections of either saline (controls) or clozapine-N-oxide (CNO, to induce membrane hyperpolarization and neuronal silencing). mOFC inactivation using this method again produced an impairment in sPIT and outcome devaluation. Together, these results suggest that mOFC governs the use of outcome representations when outcomes are currently unobservable. An implication of this is that mOFC lesioned rats should be unable to learn inhibitory associations. This was tested in a final study in which rats with sham or electrolytic mOFC lesions were first trained to form inhibitory S-R-Ø associations, and then excitatory S-R-O associations using the same stimuli, responses, and outcomes. Test performance suggested that Sham animals learned both types of association, but that lesioned animals learned only excitatory associations. Test performance further suggested that only Sham animals were able to partition each type of association into a kind of internal context or 'state'. This suggests that the mOFC not only governs the use of outcome representations, but that this information is critical to allowing animals to partition observations into states and thus make sense of the causal structure of their environment.

Disclosures: L.A. Bradfield: None. **M. van Holstein:** None. **B.W. Balleine:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: UIC Chancellor's Graduate Fellowship

DA027127

Title: Connectivity between medial prefrontal cortex and nucleus accumbens is necessary for restraint of impulsive reward-directed behavior

Authors: *K. MANSON, J. D. ROITMAN
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Abstract: Environmental stimuli that are associated with rewards often prompt approach and consummatory actions that are difficult to override, even when restraint may lead to beneficial outcomes in the short- or long-term. Many studies have elucidated the neural substrates

responsible for the drive to approach rewarding stimuli. More specifically, activation of the medial prefrontal cortex (mPFC) has been implicated in the control of reward/value-based choices and dysfunction of the PFC has been extensively associated with impulsive actions. Control of such goal-directed behavior may be executed through mPFC connections with the nucleus accumbens (NAc); the NAc integrates information about environmental cues and rewards to influence motor behaviors via basal ganglia circuitry. It is not known, however, whether mPFC projections to the NAc are also involved when an organism restrains approach behavior in the face of rewarding stimuli. We tested whether the projection from mPFC to NAc is necessary for successful behavioral inhibition in an Impulsivity task. Rats (n=10) were trained to perform a novel variant of a Go/NoGo task, which paired two distinct environmental cues with reward availability while requiring different behaviors (approach or restraint) to obtain the reward. In the task, both correct Go trials and correct NoGo trials were rewarded, and all errors were followed by a 40s time-out. We implanted infusion cannulae bilaterally in the mPFC and NAc of each animal and pharmacologically inactivated neurons in two different conditions: bilateral inactivation of mPFC (125 ng baclofen and 125 ng muscimol in 0.5[[Unsupported Character - Symbol Font ]]l) and a disconnection condition that combined inactivation of unilateral mPFC (same doses) and contralateral NAc (87.5 ng baclofen and 87.5 ng muscimol in 0.5[[Unsupported Character - Symbol Font ]]l). We then characterized accuracy in the Go/NoGo task on Go and NoGo trials separately. We found that both inactivation of mPFC and disconnection between mPFC and NAc did not affect Go accuracy, but selectively reduced accuracy on NoGo trials. That is, rats were not able to restrain their urge to press the lever even when it was disadvantageous to do so. These findings further strengthen the notion the communication between mPFC and NAc is necessary to restrain impulsive actions.

Disclosures: K. Manson: None. **J.D. Roitman:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.02. Animal Cognition and Behavior

Support: Grant in Aid for Scientific Research 19500262

Title: Adaptive contribution of the primate medial prefrontal cortex to selection, retention and usage of tactics to decide action

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Abstract: An important aspect of flexible, adaptive behavior is the use of diverse tactics to decide the action. What is the neural mechanism to select the tactics, and how different is it from the mechanism to decide action per se? Here we studied the neuronal activity of the posterior medial prefrontal cortex (pmPFC) of primates while they performed a task which required the selection of tactics either prior to or concurrently with the selection of action. We found that the pmPFC neurons encoding tactic during its selection, retention and retrieval belonged to mostly segregated populations. Similarly, separate populations of neurons encoded action during the various stages of its processing. Further, the strength of neuronal representation of tactics and action was profoundly affected by the presence of their advance knowledge. These findings indicate that the pmPFC contains diverse classes of neurons that adaptively participate in encoding, retention and retrieval of tactic and action.

Disclosures: Y. Matsuzaka: None. **J. Tanji:** None. **H. Mushiake:** None.

Poster

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Support: NRF-2013H1A8A1003842

Title: Anterior cingulate cortex is required for go/no-go avoidance performance in mice

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Abstract: A go/no-go task requests subjects to execute or to inhibit a voluntary action in response to a series of distinct sensory cues. The task is widely used for examining the ability of associative learning and sensory discrimination using rodent models. The task is also thought to require neural activity of prefrontal cortices (PFCs) in various species, since chemical lesion of PFC prevents task learning and specific units of PFC are activated during the task. Neuroimaging studies using primates reported that the anterior cingulate cortex (ACC) among PFCs is activated when subjects perform various forms of go/no-go tasks. In contrast to primates, however, it has

been not clear if ACC is involved in go/no-go task performance in rodents. A previous study using rats insisted that ACC was not required for go/no-go task learning, since pre-training lesion of ACC did not impair learning of the task. Distinct from the study, we questioned if ACC was activated in and required for go/no-go task performance after training in mice. We first confirmed that ACC was activated when subjects performed a go/no-go avoidance task, through measurement of immediate-early gene (IEG) expression. To test if ACC was required for go/no-go task performance, we ablated bilateral ACC after training and observed a severe impairment of the performance after lesion. To check if ACC was also required for other avoidance tasks, we repeated the lesion experiment using mice which performed simple avoidance tasks, but the performance was not impaired after ACC lesion. Together, our result shows that ACC is activated when mice perform the go/no-go avoidance task and required for the task performance after learning. We are currently examining the effect of activation or silencing of ACC during go/no-go task performance using optogenetic strategies.

Disclosures: **J. Jhang:** None. **J. Oh:** None. **J. Han:** None.

Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: PRESTO/JST

KAKENHI 25135736, 26282221

IRP/NIMH

Title: *In vivo* PET imaging of the behaviorally active designer receptor in macaque monkeys

Authors: Y. NAGAI[1], E. KIKUCHI[1], W. LERCHNER[2], K.-I. INOUE[3], A. OH-NISHI[1], H. KANEKO[1], Y. KATO[1], Y. HORI[1], B. JI[1], K. KUMATA[1], M.-R. ZHANG[1], I. AOKI[1], T. SUHARA[1], M. TAKADA[3], M. HIGUCHI[1], B. J. RICHMOND[2], *T. MINAMIMOTO[1,4]
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Abstract: DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) are pharmacogenetic agents that inhibit (or excite) activity of all neurons expressing the DREADD

when expressed on neuronal cell membranes and activated through systemic delivery of the targeting drug. Using the hM4Di receptor, an inhibitory DREADD that can be activated by clozapine-n-oxide (CNO), we were able to (1) monitor the location and intensity of receptor expression by *in vivo* PET-imaging, and (2) modify monkey's behavior reversibly. In our experiments, a lentiviral vector expressing the hM4Di receptor was injected into the putamen of two macaque monkeys. PET imaging using a ligand targeting the receptor showed a focal patch of high uptake at the injection site. The high-uptake region matched the site of neuronal hM4Di receptor expression identified histochemically post-mortem. Measuring uptake of the PET ligand following different CNO doses yielded to estimate the dose-occupancy relationship for binding of CNO to the hM4Di receptor. To assess the behavioral effect, an adeno-associated viral vector expressing the hM4Di receptor was injected bilaterally into the ventral striatum of a monkey that had been trained to perform a reward-size task. Our PET imaging verified the expression of the hM4Di receptor. The monkey's performance was altered by CNO treatment in a manner similar to that seen after bilateral inactivation of the ventral striatum with muscimol in two other monkeys. Given that PET imaging is capable of monitoring *in vivo* DREADD expression, the DREADD provides a potential tool to explore the neural mechanism underlying higher brain functions in nonhuman primates and, also, contributes to the development of therapeutic approaches to human neuropsychiatric disorders.

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Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: NIMH IRP

Title: Reversible DREADD inactivation of orbitofrontal cortex neurons in rhesus monkeys with contralateral rhinal cortex removal disrupts cued reward discrimination. I. Behavioral analysis

Authors: *M. A. ELDRIDGE[1], W. LERCHNER[1], T. MINAMIMOTO[2], R. C. SAUNDERS[1], B. J. RICHMOND[1]
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Abstract: Rhinal cortex (Rh) is essential to stimulus-reward association learning in monkeys. Orbital prefrontal cortex (OFC) is essential to relative value judgments. Thus disrupting the connections between Rh and OFC ought to produce a performance impairment in a task that requires both stimulus-reward association and comparisons between relative values. Two monkeys received unilateral Rh aspiration lesions. They were then trained to perform a visually-cued reward size task. At the beginning of each trial, a visual cue signaled the amount of reward (1, 2, 4 or 8 drops) available for correctly detecting when a red visual target turned green. The reward size in any trial was picked at random, with equal probability. Although the identity of the cue was irrelevant to the successful completion of the red-green color discrimination, the error rates of the monkeys decreased with increasing drop size (GLMmixed; reward size, $z = 12.74$, $p < 10^{-15}$), and were indistinguishable from unoperated controls. The operant demands were trial invariant, i.e., perform a sequential red-green color discrimination, so we interpret the differences in performance across reward size as reflecting the subjective valuation of the expected reward by the monkey as signaled by the cue. The orbitofrontal cortex contralateral to the hemisphere with the rhinal cortex removal was injected with a modified lentiviral vector expressing a Gi-coupled receptor, hM4Di, (DREADD - Designer Receptor Activated by Designer Drug) that, when activated by systemically delivered clozapine-N-oxide (CNO), causes neuronal silencing (see accompanying poster). If effective, activation with CNO should lead to a functional disconnection of Rh from OFC. After completion of all surgical procedures, the error rates of the monkeys were indistinguishable from normal monkeys or these monkeys before the DREADD injections. In behavioral testing sessions begun with systemic injection of CNO (4-5 repetitions), there was a marked reduction in the discrimination between expected reward sizes, and an overall reduction in error rate for both monkeys (GLMmixed; Monkey D: CNO*reward_size, $p < 10^{-3}$, CNO, $p < 10^{-5}$; Monkey S: CNO*reward_size, $p < 0.005$, CNO, $p < 10^{-11}$). These results demonstrate that the CNO-DREADD system can be effective for altering behavior when applied to old world monkey cortex. The reward deficit seen after a Rh-OFC disconnection could be due to an inability to act on already learned reward relationships - the monkeys are only impaired on the days with CNO treatment, whereas they respond normally to cues predicting forthcoming rewards on the days before and after the CNO treatment.

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Poster

462. Prefrontal Cortex II

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Topic: F.03. Motivation and Emotion

Support: NIMH Intramural Research Program

Title: Reversible DREADD inactivation of less than 10% of Orbitofrontal cortex neurons in interconnection with rhinal cortex is sufficient to disrupt cue discrimination in monkeys

Authors: *W. LERCHNER[1], M. A. G. ELDRIDGE[1], R. C. SAUNDERS[1], H. KANEKO[2], M. HIGUCHI[2], T. MINAMIMOTA[2], B. J. RICHMOND[1]
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Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are G-protein coupled receptors activated by clozapine-N-oxide (CNO), a ligand without other known receptor interactions. Neurons expressing DREADDs in the mammalian brain can be inhibited or excited (depending on the DREADD) by systemic delivery of CNO, independent of regional location. The hM4Di receptor is a DREADD that inhibits neuronal firing on binding of CNO, via the Gi/o pathway. We created a lentivirus-construct with a human synapsin promoter driving expression of the hM4Di receptor including a CFP fusion protein (hM4Di-CFP). Using a handheld syringe we injected the resulting lentivirus into monkey orbitofrontal cortex (OFC) of one hemisphere in each of two monkeys that already had a contralateral rhinal cortex (Rh) removal. As shown in the accompanying poster, activation of the DREADD with parenterally delivered CNO did not have any effect on monkeys with unilateral Rh lesions, whereas there was a significant change in behavior in the two DREADD expressing monkeys on the day of activation. We used antibody staining against the DREADD expressed by the lentivirus to identify the areal coverage with cellular expression of hM4Di-CFP on a flattened representation of OFC. Expression in any layer was projected onto a line running through layer 4. Our approximately 50 punctate injections led to expression within at least some layers in about 8% of the OFC. The expression widths for individual injections varied from 0.3 - 3.0 mm. Preliminary analysis using confocal microscopy shows co-expression of the DREADD with the neuronal maker NeuN in greater than 80% of neurons in areas between 0.2 - 2 mm surrounding the injection tracks (high-density expression areas). Outside the high-density areas neuronal somatic expression fell sharply. For many injection sites a bloom of fibers expressing the DREADD was visible for several millimeters around the somatic expression areas. We did not observe any

cortical layer preference of neuronal expression. We also tested the functionality of our DREADD lentivirus vector by transducing mouse primary neuron cultures. Introducing CNO into the culture bath significantly decreased neuronal spontaneous discharge. Washout of the CNO with normal culture medium was followed by a restoration of neuronal activity. In our Rh-OFC disconnection design, i.e., unilateral rhinal removal with contralateral OFC DREADD neuronal silencing, it appears that inhibition of fewer than 10% of OFC neurons, spaced in an irregular lattice throughout the OFC, is sufficient to disrupt cued reward discrimination behavior in monkeys reversibly.

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Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: F32-DA030831

R01-MH073689

NSF-DGE-1443116

Title: Transient inactivation of hippocampal-prefrontal circuitry impairs flexible spatial learning in rats

Authors: *K. M. SEIP-CAMMACK, K. G. GUISE, M. SHAPIRO
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Abstract: Learning, to be useful, must integrate relevant environmental cues, internal goals, and the history of past choices in similar situations. These processes are largely dependent upon bidirectional interactions between the hippocampus (HIPP) and medial prefrontal cortex (PFC), which help integrate new information with long-term memory to guide flexible behavior. The present study investigates whether intact PFC/HIPP circuits are required to shift flexibly between distinct behavioral responses in a single environment, by testing rats on a rapid serial spatial reversal task that requires rats to continuously monitor and update a spatial response. Rats were implanted with four cannulae, two aimed bilaterally at PFC (prelimbic/infralimbic regions) and

bilaterally at dorsal HIPP. Spatial learning was tested in a plus-shaped maze with two start arms (North, South) and two goal arms (East, West). Each rat was trained to find food reward at the end of one goal arm from pseudorandomized start arms. After reaching criterion performance (10 out of 12 correct choices), the goal arm was changed and the rat was trained to find food on the opposite goal arm until reaching criterion. Spatial reversals continued for a total of 64 daily trials. Microinfusions of the GABAA agonist muscimol were used to transiently inactivate (a) contralateral PFC/HIPP, (b) bilateral PFC, (c) bilateral HIPP or (d) ipsilateral PFC/HIPP. We hypothesized that, if disrupting PFC/HIPP communication impairs rats' ability to respond flexibly to changing contingencies in the same environment, then contralateral and bilateral (but not ipsilateral) inactivations should impair rats' ability to learn serial spatial reversals. Muscimol infusions into contralateral PFC/HIPP, bilateral PFC or bilateral HIPP impaired rats' overall performance during the session (<80% correct). Bilateral PFC inactivation spared learning of the initial association but profoundly impaired reversal learning. Bilateral HIPP inactivation impaired all aspects of the spatial task, with rats rendered unable to distinguish between start and goal arms. Contralateral PFC/HIPP inactivation impaired performance on the initial association more than reversals; when this combined inactivation was retested weeks later, this deficit was absent. Ipsilateral inactivations did not affect performance. The results demonstrate that interactions between mPFC and dorsal HIPP are required for spatial learning. We propose that transient impairment following crossed PFC and dorsal HIPP inactivation may be due to task encoding by other intact structures within this circuit, e.g., ventral HIPP.

Disclosures: K.M. Seip-Cammack: None. **K.G. Guise:** None. **M. Shapiro:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight endowment fund for neuroscience

Whitehall Foundation

Title: Parallel processing in the hippocampal CA1 region

Authors: *A. PEVZNER, B. J. WILTGEN
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Abstract: It is hypothesized that the hippocampus forms a contextual representation in part by integrating polymodal information from the entorhinal cortex (EC). However, projections from distinct regions of the EC are kept segregated in some parts of the hippocampus. Specifically, medial EC (MEC) heavily projects to the proximal part of CA1 (pCA1, closer to CA3) while the lateral EC (LEC) mainly inputs to distal CA1 (dCA1, closer to subiculum). Consistent with anatomical data, electrophysiological recordings demonstrate that CA1 produces unique response along the proximodistal axis. Place cells in pCA1 show more discrete firing and fewer firing fields, as well as greater phase locking to MEC theta oscillations when an animal explores an environment (Henriksen et al. 2010). In addition, place cells in dCA1 are modulated by local objects in the arena (Burke et al. 2011). These initial studies conclude that pCA1 and dCA1 show response properties consistent with the proposed role of MEC in spatial processing and LEC in object processing, respectively. The current experiments will examine the contribution that these parallel circuits make to learning and memory. To do this, we developed two version of the Barnes maze, a spatial reference memory task. In the 'spatial' version, animals must find a hidden escape box whose location is fixed relative to distal cues of the room. In contrast, in the 'object' version the escape box moves every session, so that it is dissociated from the room cues and rather its location is dictated by the local cues on the arena. These two versions of the Banes maze allowed us to interrogate the EC-hippocampal CA1 circuitry with a similar behavioral task as well as assess immediate early gene (IEG) activity. Initial studies indicate that mice are able to acquire both version of the task to a comparable degree. Furthermore, preliminary cell activity data, as assessed with the IEG c-Fos protein, points to differential engagement of pCA1 and dCA1 during the spatial version of the Barnes maze. These data illustrate the existence of two parallel circuits in the hippocampus. Subsequent experiments will use DREADD receptors to selectively silence these circuits during memory retrieval. We predict that inactivation of pCA1 will impair memory based on spatial cues while inactivation of dCA1 will impair memory that is based on the location of objects.

Disclosures: **A. Pevzner:** None. **B.J. Wiltgen:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

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Nakajima Foundation Fellowship

Title: Cortical representations are reinstated by the hippocampus during memory retrieval

Authors: *K. Z. TANAKA[1], A. PEVZNER[2], A. HAMIDI[2], B. J. WILTGEN[2]
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Abstract: The hippocampus is assumed to retrieve memory by reinstating patterns of cortical activity that were observed during learning. To test this idea, we monitored the activity of individual cortical neurons while simultaneously inactivating the hippocampus. Neurons that were active during context fear conditioning were tagged with the long-lasting fluorescent protein H2B-GFP and the light activated proton pump ArchT. These proteins allowed us to identify encoding neurons several days after learning and silence them with laser stimulation. When tagged CA1 cells were silenced, we found that memory retrieval was significantly impaired. In addition, overlap between c-Fos (expressed during retrieval) and H2B-GFP (expressed during learning) was significantly reduced in the CA1 region. C-Fos expression in H2B-GFP negative neurons was not reduced. These results indicate that ArchT stimulation selectively silenced tagged CA1 neurons during memory retrieval. Notably, reactivation was significantly correlated with freezing in control groups, but not in ArchT simulated animals. When previously active CA1 neurons were silenced during retrieval we also found that representations in the cortex (entorhinal, retrosplenial, perirhinal) and the amygdala (central) could not be reactivated. Importantly, hippocampal inactivation did not alter the total amount of activity in most brain regions. Instead, it selectively prevented neurons that were active during learning from being reactivated during retrieval. These results provide evidence that the hippocampus is fundamental for memory because it can reinstate patterns of activity that were originally present during learning.

Disclosures: K.Z. Tanaka: None. **A. Pevzner:** None. **A. Hamidi:** None. **B.J. Wiltgen:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 463.04/TT64

Topic: F.02. Animal Cognition and Behavior

Title: Redundant spatial representation along the longitudinal hippocampal axis: Overcoming an interference-generalization tradeoff

Authors: *A. T. KEINATH[1], M. E. WANG[2], J. T. DUDMAN[3], I. A. MUZZIO[1]
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Abstract: Recent work has revealed spatial scaling in place cell field size along the longitudinal hippocampal axis. Increasing place field size toward the ventral pole has been taken to suggest that the ventral hippocampus sacrifices precision to allow for representation on a large spatial scale, or does not convey spatial information at all. However, the spatial properties of individual ventral cells and ventral populations have not been thoroughly characterized. It is possible that spatial scaling may instead signal a shift from sparse to distributed spatial coding, thereby preserving the resolution of spatial information. To explore this possibility, we recorded from dorsal and ventral CA1 neurons in freely moving mice exposed to several varying visuospatial and olfactory contexts. We found that, despite their broad spatial tuning, single ventral cells show contextual and cue-dependent responses similar to those of dorsal cells. Furthermore, at the population level, we found that spatial representation was faithful across both regions. Our results indicate that spatial information is coded with high precision by both dorsal and ventral populations, implying a shift in coding rather than a shift in content. With support from neural network modeling, we suggest that this redundant spatial representational gradient may allow the hippocampus to overcome the conflict between memory interference and generalization inherent in neural network memory, biasing ventral population activity toward generalization across locations.

Disclosures: A.T. Keinath: None. **M.E. Wang:** None. **J.T. Dudman:** None. **I.A. Muzzio:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Program#/Poster#: 463.05/TT65

Topic: F.02. Animal Cognition and Behavior

Support: Mercator Stiftung

SFB 874

Title: Segregation of spatial information along the proximodistal axis of CA3: Additional hints of segregated spatial and non-spatial subnetworks within the hippocampus

Authors: V. FLASBECK, N. NAKAMURA, *M. SAUVAGE
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Abstract: We have recently brought evidence of a proximodistal functional segregation of CA3 by reporting that the proximal part of CA3 (close to the dentate gyrus) contributes strongly to non-spatial memory, but not its distal part (close to CA2) (Nakamura et al, Journal of Neuroscience, 2013). Based on these results and those of others, we suggested the existence of more 'direct' segregated spatial and non-spatial hippocampal subnetworks that could be recruited when only one dimension of a memory representation is relevant (spatial or non-spatial), e.g. when the association of different types of information is not required. To further test this hypothesis, here we investigate whether the distal part of CA3 is more involved in spatial memory than its proximal part. To compare readily our results with those of our previous study (Nakamura et al, 2013), we developed a new behavioral memory task with matching experimental conditions (a delayed non-matching to sample task with a study list of 10 stimuli, a delay of 20 min and a list of 20 test stimuli) but based this time on spatial information instead of non-spatial ones. Briefly, in this 24-arm maze (the 'centipede'), the memory for spatial contexts located outside of the arms was tested after rats were exposed to a subset of them. This task was combined to a high-resolution molecular imaging technique based on the detection of the expression of the immediate-early gene Arc, used as a marker of neuronal activation, to evaluate whether spatial information was differentially represented along the proximodistal axis of CA3. We found that distal CA3 was indeed more recruited than proximal CA3 during the recognition phase of this spatial task, revealing that spatial information is segregated along the proximodistal axis of CA3. These results bring further support to our hypothesis of the existence of dedicated spatial and non-spatial hippocampal subnetworks that could be preferentially recruited when either the spatial or the non-spatial dimension of a memory representation is relevant.

Disclosures: V. Flasbeck: None. **N. Nakamura:** None. **M. Sauvage:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Program#/Poster#: 463.06/TT66

Topic: F.02. Animal Cognition and Behavior

Title: The endocannabinoid system differentially modulates spatial memory retrieval depending on stress level at encoding

Authors: *M. MORENA[1,2,3,4], A. PELOSO[4], M. J. GRAY[1,2,3], V. TREZZA[6], M. N. HILL[1,2,3], P. CAMPOLONGO[4,5]
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Abstract: The level of stress associated to the environmental condition differentially affects spatial memory processes in rats1,2. We have previously shown that variation in environmental aversiveness differentially influences cannabinoid effects on memory processes in rats3,4 and that glucocorticoids interact with the hippocampal endocannabinoid system in impairing contextual memory retrieval5. Here we investigated the role of the hippocampal endocannabinoid system on spatial memory retrieval in rats trained under two experimental conditions which differed with respect to their training associated stress levels. Male adult Sprague Dawley rats were trained in a Morris Water Maze task at two different water temperatures (19° C and 25° C) in order to elicit different levels of stress2. To test cannabinoid effects on spatial memory retrieval, the synthetic cannabinoid agonist WIN55,212-2, the AEA hydrolysis inhibitor URB597 or the 2-AG hydrolysis inhibitor JZL184 were bilaterally infused into the dorsal hippocampus 1 hr before the retrieval (probe) trial in separate groups of animals. We found that WIN55,212-2 impaired memory retrieval only in rats trained under more stressful conditions (19° C). Such effect was blocked by administration of the CB1 antagonist AM251. Interestingly, URB597 did not alter spatial memory retrieval performances in any of the two experimental conditions. However, highly comparable with WIN55,212-2 effects, JZL184 impaired spatial memory retrieval only in rats trained under higher stressful conditions via an interaction with CB1 receptors. Consistently, rats trained under higher stress showed an increase in hippocampal 2-AG levels, both after the training and probe trials, and alterations in CB1 affinity and in the activity of the main 2-AG degradative enzyme MAGL after the probe trial than rats trained under less stressful conditions. The present findings indicate that the hippocampal endocannabinoid system plays a central role in mediating stress effects on spatial memory retrieval, shedding light on the neurobiological mechanism involved in the differential impact of stress on memory processes. 1Akirav et al., Learn Mem. 11 (2004) 188-195; 2Salehi et al., Learn Mem. 17 (2010) 522-530; 3Campolongo et al., Front Behav Neurosci. 20 (2012) 11; 4Campolongo et al., Neuropsychopharmacology. 38 (2013) 1276-1286; 5Atsak et al., Proc Natl Acad Sci U S A. 109 (2012) 3504-3509.

Disclosures: **M. Morena:** None. **M.J. Gray:** None. **M.N. Hill:** None. **A. Peloso:** None. **V. Trezza:** None. **P. Campolongo:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Program#/Poster#: 463.07/TT67

Topic: F.02. Animal Cognition and Behavior

Title: Membrane potential dynamics of dorsal hippocampal CA1 neurons during exploration of familiar and novel virtual environments

Authors: *J. D. COHEN, A. K. LEE
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Abstract: The hippocampus, including dorsal CA1, is necessary for the behavioral expression of memory following experiences in a novel context. Indeed, many neurons in CA1 show immediate or rapid changes in spiking activity on the first exposure to novel items and places. These changes are thought to reflect the cellular and network level processes associated with long-term contextual memory formation. However, little is known about the subthreshold activity underlying the dynamic suprathreshold spiking observed during novel exploration and memory formation. To reveal this activity, and compare it to that expressed in more familiar contexts, we performed whole-cell patch-clamp recordings of dorsal CA1 neurons as animals explored familiar and novel environments. Experiments were performed on head-fixed adult mice (n=27, 8-12 wk, 20-30 g), trained to navigate on a large air-cushioned spherical treadmill (16” diameter, 70 g) to seek sweet water rewards in visual-based virtual environments. Animals were trained to explore several visually distinct and geometrically unique linear tracks (“worlds” included straight and bent hallways, ovals, and figure-8 shapes), which after several days of exposure became familiar environments. On recording days, trained animals were either placed in a familiar world or exposed to a unique novel world (n=62 putative pyramidal neurons, 2 theta-modulated fast-spiking interneurons). If conditions permitted, animals were subsequently exposed to additional familiar and/or novel worlds (n=48/62). We present evidence based on behavioral metrics, such as licking and running trajectory, demonstrating that mice can respond to and rapidly learn about novel places in virtual reality. Consistent with previous studies, some cells expressed robust changes in suprathreshold spiking activity between different environments (remapping). Interestingly, many cells also showed subthreshold membrane potential dynamics that did not lead to changes in suprathreshold spiking behavior. Most often this was the case with silent cells (those that did not produce spiking during spatial exploration). Finally, we present and contrast an array of statistical results extracted from the continuous subthreshold membrane potential and suprathreshold spike train records of more active neurons (n=54 worlds in which

cells expressed place field activity) recorded in familiar (n=37) and novel worlds (n=17), and the transitions between them.

Disclosures: J.D. Cohen: None. **A.K. Lee:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH-58846

NIH Grant T32-HD071845

Title: Sparing of object and spatial recognition memory in adult monkeys with neonatal perirhinal lesions

Authors: *A. R. WEISS, J. BACHEVALER
Emory University/YNPRC, Atlanta, GA

Abstract: The contribution of the perirhinal cortex (PRh) to recognition processes is well characterized in adults. Damage to the medial temporal lobe (MTL) that includes the PRh produces object recognition deficits more severe than combined damage to the hippocampus and other MTL structures excluding the PRh (Zola-Morgan & Squire, 1985; Meunier, et al., 1993; Meunier et al., 1996). Yet, the same lesions have limited effect on recognition of spatial locations (Nemanic et al, 2004; Aggleton, 2004). In a recent longitudinal study, we assessed whether the same outcomes will follow neonatal perirhinal (Neo-PRh) lesions. We demonstrated that Neo-PRh lesions impaired object recognition memory using a Visual Paired Comparison (VPC) task. As compared to controls (Neo-C), the deficit in Neo-PRh animals emerged as early as 1.5 months, became more pronounced in adolescence (18 months), and remained present in adulthood (48 months) (Zeamer et al, submitted). Here, we tested object and spatial recognition memory in the same groups of monkeys as they reached adulthood, using three operant recognition tasks: Delayed Non-Match-to-Sample (DNMS) and Object Memory Span (OMS), measuring object recognition, and Spatial Memory Span (SMS), measuring recognition of spatial locations. Neo-PRh lesions did not alter performance on any of the three tasks [DNMSacq: 200 trials vs 266 trials; OMS: 4.09 vs 4.21; SMS: 2.27 vs 2.12 for groups Neo-C and Neo-PRh

respectively]. The normal performance of animals with Neo-PRh lesions on our operant object recognition tasks (DNMS, OMS) contrasts with the severe impairment of the same animals in the object VPC task. The functional sparing of object recognition on our operant tasks suggests that the reward component of these tasks is sufficiently motivating for subjects to develop alternative strategies mediated by other cortical areas, such as the ventrolateral prefrontal cortex, to maintain cognitive representations of the test stimuli over relatively short, distraction-free, delays. Given that these alternate strategies are not present in monkeys that had acquired the PRh lesions in adulthood, the data suggest that the Neo-PRh lesions have resulted in compensatory mechanisms most likely supported by the prefrontal cortex. This work was supported by grants MH-58846 and T32-HD071845.

Disclosures: **A.R. Weiss:** None. **J. Bachevaler:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIMH 1F32MH097413-01

NIH/NIA 3 R37-AG13622:06

Title: Integration of multiple spatial memories in hippocampus

Authors: ***D. J. CAI**[1], J. L. SHOBE[1], T. SHUMAN[2], K. BAUMGAERTEL[4], J. BIANE[5], A. C. FRANK[1], M. MAYFORD[4], A. J. SILVA[3]
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Abstract: How are different episodic memories integrated in the brain? We tested the hypothesis that CREB may be a mechanism for temporally and contextually linking memories via cellular co-allocation. Previous findings in the amygdala suggest that viral CREB modulates memory allocation (i.e. biases neurons towards inclusion in the memory trace) and enhances memory consolidation. However it remains unclear how generalizable these findings are to endogenous fluctuations of CREB and hippocampus-dependent memories. We hypothesized that endogenous

CREB activated by memory acquisition could mimic the effects in hippocampus seen with viral CREB in amygdala. Thus we tested the following predictions using the transgenic TetTag system: (i) CREB activation is transiently increased in hippocampus following a spatial learning task, (ii) following an initial spatial task (similar to viral overexpression), endogenous increases in CREB activation bias the allocation of the second memory to many of the same neurons recruited to store the first memory (iii) the first memory will enhance the consolidation of the second memory (due to increased levels of CREB activation from the induction of the first memory), (iv) sharing a sub-population of the neural ensemble between two memories creates a contextual link between them. Consistent with these predictions, we found that CREB activation was increased in dorsal hippocampus 5 hours following a spatial learning task. We found that this 5-hour delay between two spatial learning episodes resulted in greater overlap between the two cellular ensembles in CA1 (i.e. co-allocation) than 7 days. Furthermore, with the 5-hour delay, we also found enhanced behavioral memory for the second task, presumably by strengthening the contextual representation. Interestingly, features from one context were generalized to the other context when the tasks were spaced 5 hours, but not 7 days, apart. These results imply that CREB may be a mechanism for how multiple memories are temporally and contextually linked.

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Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R25 MH059472

Title: Enhancing graduate and post-doctoral training: insights from the neural systems & behavior course at mbl in woods hole

Authors: *A. A. FENTON[1], R. M. HARRIS[2], H. A. HOFMANN[3]
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Dept. of Integrative Biology, Inst. for Cell. and Mol. Biol., [3]Dept. Integrative Biology, Inst. for Cell. & Mol. Biology, Inst. for Neurosci., The Univ. of Texas at Austin, Austin, TX

Abstract: The Neural Systems & Behavior (NS&B) course has provided intensive training in the concepts and methodology of behavioral neurobiology and systems neuroscience to outstanding pre- and postdoctoral students and junior faculty since 1978. During this eight-week summer course, within a discovery-driven curriculum, 20 trainees receive intensive lectures and discussion, one-on-one interaction with internationally renowned scientists, and extensive hands-on laboratory training with a variety of invertebrate and vertebrate preparations using state-of-the-art techniques and equipment. More than 30 dedicated faculty members arrive from all over the world to teach in NS&B and at least another 7 visit for 1-2 days. They present many different approaches to investigating the neural basis of behavior. NS&B trainees learn to think creatively about the brain, behavior, representation of information and plasticity. Each year, the trainees are exposed to at least 7 different preparations. These "modules" include rodent somatosensory cortex, rabbit cerebellum, mouse hippocampus, the brains of songbirds, weakly electric fish, the spinal cord of zebra fish, the crab stomatogastric ganglion, and the nervous systems of the fruitfly, the nematode Caenorhabditis elegans, and the medicinal leech. Methodologies incorporate intracellular and extracellular electrophysiology, imaging, biomechanics, computational modeling, and molecular biology. Trainees attend all lectures but focus on four modules, each for a 2-week cycle. One week of each cycle is devoted to trainee-developed discovery research, which has resulted in peer-reviewed publications. There is an explicit effort to teach and conduct research across multiple levels of biological organization. This diversity of approaches provides students with a global perspective on the problems underlying the relationship between brain and behavior. NS&B provides a novel scientific perspective with its blending of methodologies, intellectual traditions, and experimental preparations, and thus adds exceptional value to graduate and post-doctoral training in neurobiology and systems neuroscience. Most (>97%) PIs of the last 5 years of NS&B trainees strongly agree/agree that the trainees' participation in the course was valuable and has a positive impact on the PI's research program. The training and professional social networks that NS&B provides has the capacity to catapult the scientific careers of trainees and has established NS&B as the premier short course for training the next generation of neuroscientists.

Disclosures: **A.A. Fenton:** None. **R.M. Harris:** None. **H.A. Hofmann:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: MH084038

MH099128

Title: Differential effects of acute psychotomimetic NMDAR antagonists on hippocampal subfields

Authors: *H. JOURDI[1], H.-Y. KAO[2], K. W. TUNNELL[2], D. DVORAK[3], E. LESBURGUÈRES[2], A. A. FENTON[2]
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Abstract: PCP, an NMDAR antagonist, elicits psychotomimetic effects in drug users and in animal models of mental illnesses. We have shown that acute PCP treatment *in vivo* elicits neural discoordination and cognitive control impairment that are observed at the electrophysiological and behavioral levels, respectively. PCP increased fast γ oscillations in hippocampus (HPC) local field potential but not slow γ oscillations, suggesting differential effects within HPC synaptic domains. Because we find that PCP's effects on behavior and electrophysiology are rapid and can be abrogated by inhibiting protein synthesis, we decided to investigate the activation of the protein translation machinery (AKT, mTOR, 4EBP) in *in vitro* treated HPC slices using western blotting. We also compared the effects of PCP to other NMDAR antagonists on the translation machinery and actin cytoskeletal assembly. Our findings indicate that the protein translation machinery was activated within minutes by PCP and this was accompanied by increased expression of ARC. In addition, the activity of actin cytoskeleton regulatory proteins was altered, including the phosphorylation of cofilin and its regulatory proteins. Since the current literature excludes a description of which NMDAR subunit is predominantly affected by PCP and how these effects vary across HPC subfields, we are exploring the effects of NMDAR subunit-specific antagonists on the same pathways. Comparing the effects of PCP and NMDAR subunit-specific antagonists should reveal both subfield- and subunit-specific contributions of these drugs to the alterations of the HPC information processing circuit that is caused by PCP.

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Poster

463. Animal Models: Spatial Learning and Place Cells

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NSF- IOS 1326187

Title: Integrated molecular and synaptic hippocampal mechanisms of place avoidance learning and memory

Authors: *R. M. HARRIS[1], J. M. ALARCON[2], H. A. HOFMANN[1], A. A. FENTON1[3]
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Abstract: A contemporary systems neuroscience understanding of brain and behavior relationships increasingly benefits from incorporating data across levels of organization.. Here, we investigated the neuromolecular basis of hippocampal learning and memory formation within an explicitly integrative framework. We evaluated the hypothesis that memory is based on long-term changes in neuronal circuits via modulation of specific molecular pathways that regulate synaptic function and plasticity. This predicts pre- and post-synaptic changes in gene expression and interactions between genotype and training experience so we began to examine the transcriptional regulation of synaptic function within defined regions of the hippocampal circuit. We used an active place avoidance task in C57BL/6 wild type and Fragile X mental retardation protein (Fmr1) knockout mice to examine spatial memory. Mice received three 10 min place avoidance training trials and were then tested for memory retention 24 h later. Control animals were exposed to the identical apparatus but never conditioned. Behavior was quantified using automated video tracking. After the memory retention test, we killed the mice and prepared *ex vivo* hippocampus slices for electrophysiological investigation of potential changes in the synaptic function of CA1 neurons as a consequence of place avoidance learning. Using alternate slices, we obtained tissue punches of CA1 and CA3 regions to perform real-time PCR to detect potential changes in mRNA expression levels of key genes implicated in functional synaptic changes. Our candidate genes represent an array of mechanistic stages considered relevant for the acquisition and storage of memory, including: N-ethylmaleimide sensitive fusion protein (nsf), ionotropic and metabotropic glutamate receptors (gria1, grim1), N-methyl-D-aspartate receptor 1 (grin1), post-synaptic persistent kinases (camk2d, prkcz), transcription factors (creb1, fos), and the translation repressor fragile X mental retardation protein 1 (fmr1). Multivariate statistical analyses of trained and untrained wild type males revealed that nsf, gria, grin, and camk2d form a functional module associated with locomotor activity and synaptic plasticity in

CA1, while prkcz expression in CA1 covaries with behavioral measures of memory linking to the functional module for locomotor activity. Our results demonstrate how the integration of quantitative behavioral assays, *in vitro* slice physiology, gene expression analysis, and multivariate statistical analyses provides novel insights into the multilevel processes involved in hippocampal learning and memory.

Disclosures: R.M. Harris: None. **J.M. Alarcon:** None. **A.A. Fenton1:** None. **H.A. Hofmann:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: R01MH084038

R01MH099128

Title: Discoordination of cross-frequency coupling in animal models of cognitive dysfunction

Authors: *D. DVORAK[1], B. RADWAN[3], H.-Y. KAO[3], A. A. FENTON[3,2]
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Abstract: Despite diverse etiology, there is substantial overlap in the symptoms of cognitive dysfunction associated with mental illness and disability. We examined the hypothesis that core symptoms like cognitive inflexibility and control failures arise from a common pathophysiology. Here we focus on the cross-frequency coupling (CFC) of local field potential (LFP) oscillations because, in healthy brains, CFC may be a mechanism for organizing computational resources, segmenting distinct streams of information, and effecting information transfer between neural circuits and across memory processes. The dynamics of CFC may provide a means to robustly respond to sensory, motor and cognitive events while allowing enough flexibility for prioritizing ongoing cognitive demands. The discoordination hypothesis asserts that abnormal CFC dynamics in neural oscillations is a common pathophysiology for cognitive impairment. We compared CFC in Fmr1 knockout (KO) mice and rats acutely treated with phencyclidine (PCP), models of dysfunction that are relevant to fragile X syndrome (FXS) and schizophrenia. We

focus on the phase-amplitude coupling (PAC) of gamma (30-100 Hz) oscillation amplitude and theta (4-12 Hz) oscillation phase in the LFP of hippocampus CA1. Baseline recordings were made during unconditioned exploration of circular open field arenas and during cognitive effort in an active place avoidance task that challenged rodents to avoid a shock zone on the arena while it rotated to dissociate spatial cues into two streams of information, one stationary and one rotating. Prior to training, PAC was greater in Fmr1 KO mice than wild type (WT) controls and decreased during the session in both genotypes. KO and WT mice learned the avoidance equally well but were impaired when the shock location was changed or removed. This cognitive inflexibility coincided with exaggerated PAC between theta and fast gamma (60-100 Hz) oscillations. Place avoidance was impaired after PCP was administered to rats, despite performance being optimal before the drug. Cognitive impairment also coincided with exaggerated PAC between theta and fast gamma. These findings are consistent with the prediction that cognitive impairment of diverse etiology arises from common pathophysiology, in this case, excessive PAC between fast gamma and theta oscillations in CA1. This may unbalance the intra- and extra-hippocampal inputs that control the circuit output. Finding a unified measure and a common set of distinguishable pathophysiologies would have enormous value for objectifying and differentiating the diagnoses of mental dysfunction in heterogeneous disorders like FXS and schizophrenia.

Disclosures: **D. Dvorak:** None. **B. Radwan:** None. **H. Kao:** None. **A.A. Fenton:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Title: Schemas in the hippocampus: A hierarchical Bayesian approach to one-trial learning and time dependent memory consolidation

Authors: *A. JOHNSON[1], M. HASSELMO[2], P. SCRHATER[3]
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Abstract: The hippocampus plays a critical role in spatial look-ahead, single-trial learning, memory consolidation, and imagination. Each of these learning dynamics depends on memory schemas. We have developed a hierarchical Bayesian approach that learns the statistical structure

of behavioral tasks in order to predict future task observations. We hypothesize that hierarchical structure learning corresponds to learning task schema. We show how such schema-based structure learning can account for hippocampal dependence of single trial learning and variable time memory consolidation on the paired associate task (Tse et al., 2007). New paired associate learning, informed by previous learning about the task contingencies embedded within task schemas, facilitates learning and allows rich inferences from single observations. Memory consolidation and hippocampal independence of a predictive task representation is hypothesized to be dependent on the stability of the representation. We show how task representations in well-structured tasks achieve stability very quickly and consequently move into an easily consolidated form. Using this model, we replicate the single trial learning and fast memory consolidation findings from Tse et al. (2007). Structure learning associated with task schemas provides the basis for a generalized prediction error signal. In contrast to reward prediction signals in reinforcement learning, the prediction error signal in the schema learning framework indicates the consistency of an observation with a given representation. The time course of prediction error signals is unique to each potential representation and can be used to infer the representation an individual animal is using to solve a task. We show how these prediction errors correspond to spontaneous exploration within intrinsically motivated learning tasks.

Disclosures: **A. Johnson:** None. **M. Hasselmo:** None. **P. Scrhater:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: ERC grant 207807

Title: Septal modulation of hippocampal learning and memory

Authors: ***L. MAGNO**[1], C. SCHMIDT-HIEBER[1], C. BARRY[2], Y. MILNER[1], P. THEODOTOU[1], A. N. RUBIN[1], M. HAUSSER[1], N. KESSARIS[1]
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Abstract: Learning and memory processes are dependent on hippocampal function. Several neuromodulatory systems are known to innervate the hippocampus and regulate its activity. One

of these is the cholinergic projection system from the septum. A prominent role of central acetylcholine in cognitive functions has been demonstrated through studies in mice, non-human primates and humans: potentiating cortical cholinergic signalling promotes mnemonic functions, whilst damaging the projections or blocking the cholinergic receptors leads to cognitive impairment and deficits in memory acquisition. However, defining the precise role of cholinergic septal neurons in the modulation of hippocampal circuitry and function has been more challenging, as pharmacological manipulation of the septal cholinergic system has resulted in variable and conflicting findings. To investigate the role of the septal cholinergic innervation of the hippocampus we took advantage of the cholinergic neurons' requirement for the transcription factor Nkx2-1. We used a conditional genetic strategy in mice to inactivate Nkx2-1 in the septum, thus affecting selectively the septo-hippocampal cholinergic projection system. We demonstrate specific loss of septal cholinergic markers and denervation of the hippocampal formation. Mutants are viable and fertile, and when subjected to preliminary behavioural screening do not show significant deficits in sensory and motor functions. However, in specific learned and innate hippocampus-mediated behaviour (learned: delayed non match-to-sample T-maze task and novel object recognition task, innate: nesting task) mutants show a clear impairment. Our findings provide direct evidence for an essential role of the cholinergic system in hippocampal mnemonic and cognitive functions.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Comparing dorsal and ventral hippocampus oscillations during learning

Authors: *J. YOON[1], G. N. NEWMAN[2], R. A. JACKSON[2], X. LI[2], S. P. VU[2], A. DHURI[2], E. J. MARKUS[2]
[1]Psychology, [2]Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: It is postulated that the oscillatory dynamics in the hippocampus support cognitive function in humans and rodents. Theta (4-12 Hz), the most prominent oscillation in the hippocampus, has been linked to both spatial navigation and mnemonic processes. In most

studies theta oscillations were recorded from the dorsal hippocampus. However the hippocampus is not a homogenous structure, anatomical and functional dissociations exist along the dorso-ventral axis. Theta oscillations were simultaneously recorded from dorsal and ventral hippocampus in rats while they learn "place" and "response" tasks on a plus maze. For the place task, rats were rewarded for going to the same "place" regardless of the start arm. For the response task rats were rewarded for making a right turn on the maze. These tasks differ in the degree they depend upon hippocampus. For each task, theta frequency, coherence, power and it's correlation with running speed were compared during the choice and post reward running epoch. How theta power and coherence were modulated with learning was also analyzed, both within and across the test days.

Disclosures: J. Yoon: None. **G.N. Newman:** None. **R.A. Jackson:** None. **X. Li:** None. **S.P. Vu:** None. **A. Dhuri:** None. **E.J. Markus:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

Location: Halls A-C

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Program#/Poster#: 463.17/TT77

Topic: F.02. Animal Cognition and Behavior

Title: Temporal sequence learning by rats in a radial arm water maze

Authors: *S. LEE[1], B. TIMMERMAN[2], V. WICKENHEISSER[2], S. PATEL[2], E. J. MARKUS[1]
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Abstract: The hippocampus has been linked with the formation and retrieval of an experience or episodic memories. To date, most rodent studies have focused on the "what" and "where" aspects of hippocampal processing. Our study examines the "when" aspect through teaching rats a temporal sequence task. Male F-344 rats (N=6), approximately six months old, were trained in an eight-arm radial water maze with removable escape platforms. Rats were taught the first arm in the sequence, and additional arms were added when rats mastered the previous arm(s). After initial exposure to the maze and task, swim latencies stabilized to five seconds for a rat to select their first arm choice. Rats successfully selected the correct goal location in less than one-minute. The animals had multiple maze sessions separated by short inter-session intervals (<10min). Each session had a different fixed correct goal arm; however the room and maze were kept identical requiring the use of the session order to identify the correct goal. With training, animals could reliably learn a 6+ session sequence, with each session taking under 10 seconds. While this

has previously been shown on a "win-shift" appetitive task, to our knowledge this is the first time rats have been shown to master a 6+ sequence in an avoidance paradigm. The degree to which this process is dependent on the hippocampus will be explored.

Disclosures: S. Lee: None. **B. Timmerman:** None. **V. Wickenheisser:** None. **S. Patel:** None. **E.J. Markus:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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NIMH/NRSA T32 MH020002-13 Training Program in Cognitive Neuroscience

Temporal Dynamics of Learning Center

Medical Research Service of the Department of Veterans Affairs

Title: Medial entorhinal cortex lesions disrupt hippocampus-dependent place memory in rats

Authors: *J. B. HALES[1], S. SATURDAY[1], S. LEUTGEB[2,3], L. R. SQUIRE[6,4,5], R. E. CLARK[1,6]
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Abstract: Memory processing and spatially selective neuronal firing are both known to occur in the hippocampus. Spatially selective firing has also been recorded in the medial entorhinal cortex (MEC), an adjacent cortical region that provides major projections to the hippocampus. We recently showed that MEC lesions in rats disrupt hippocampal place cell precision and stability. Additionally, when MEC lesions were made pre-training, rats were impaired at acquiring the watermaze task, but were spared on other hippocampus-dependent memory tasks. Many tasks are more sensitive to hippocampal damage introduced after training rather than before training. Here we examined the role of the MEC in memory retrieval. All previous lesion studies targeting the entorhinal cortex have spared the dorsal-medial aspect of the MEC, where the most spatially

precise cells, such as grid cells, are located. We developed a method to selectively remove the MEC with minimal damage to the hippocampus. The novelty of our approach was that we targeted our lesion along the plane of the MEC cell layers. We then examined memory retrieval using a number of behavioral tasks designed to assess hippocampus-dependent spatial and nonspatial memory. Rats with MEC lesions were impaired at place memory retention in the watermaze task, performing no different than chance. This impairment is similar to what is found after hippocampal lesions. In contrast, rats with MEC lesions exhibited normal context fear conditioning. These results suggest that the MEC is required for the retrieval of place memory, but not for retrieval of context fear memory.

Disclosures: J.B. Hales: None. **S. Saturday:** None. **R.E. Clark:** None. **L.R. Squire:** None. **S. Leutgeb:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Support: Shota Rustaveli National Science Foundation. Dictoral grant DO/196/7-276/1

Title: Spatial long-term memory and modulation of NMDA receptor subunit expression in medial septal immunolesioned rats

Authors: *L. KRUASHVILI[1], M. MEPHARISHVILI[2], M. DASHNIANI[3], M. BURJANADZE[3], M. DEMURISHVILI[4]
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Abstract: The present study was designed to investigate the effect of selective immunolesions of cholinergic and GABA-ergic SH projection neurons (using 192 IgG-saporin and GAT-1 saporin, respectively) on spatial memory assessed in water maze and the N-methyl-D-aspartate (NMDA) receptor GluN2B subunit expression in the rat hippocampus. We used water maze training protocol with eight training trials. One day after training, probe test with the platform removed was performed to examine long-term spatial memory retrieval. We found that immunolesion of medial septal cholinergic neurons did not affect spatial learning as exhibited by a decreased

latency to find the hidden platform across the eight training trials. In contrast, rats with immunolesions of medial septal GABAergic neurons did not show a decreased latency across training trials in water maze. Trained control rats spent significantly longer than chance (15 s) performances such as swimming time in test sector (where the hidden platform was located). Moreover, they spent significantly longer in test sector than in the opposite sector, confirming the establishment of long-term memory. In contrast, the preference for test sector was abolished in medial septal immunolesioned rats. Because Saporin treated rats learned the location of the hidden platform during training, the results suggest that saporin treated rats could not remember the training a day later. We found that the expression level of NR2B subunit of NMDA receptor in the hippocampus was decreased significantly in the GAT-1 treated group compared with the control and saporin treated groups. In conclusion, our findings suggest that immunolesion of medial septal GABAergic neurons can interrupt hippocampus-dependent spatial learning, possibly through modulation of NMDA receptor subunit expression in the hippocampus. Moreover, our finding that selective lesions of medial septal cholinergic neurons affects probe-test performance but not spatial learning, suggests that septohippocampal cholinergic projections are involved specifically in the consolidation or retrieval, but not in the acquisition of long-term spatial memory.

Disclosures: L. Kruashvili: None. **M. Mepharishvili:** None. **M. Dashniani:** None. **M. Burjanadze:** None. **M. Demurishvili:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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NIH

Cisco Systems Stanford Graduate Fellowship

Title: Time-lapse imaging in freely behaving mice of CA1 hippocampal ensemble representations of associations between reward and spatial location

Authors: *M. CARR LARKIN[1,2], E. O. HAMEL[1,3], L. J. KITCH[1,4], J. LI[1], M. J. SCHNITZER[1,2,3,5]
[2]Dept. of Biol., [3]Dept. of Applied Physics, [4]Dept. of Electrical Engin., [1]Stanford Univ., Stanford, CA; [5]HHMI, Stanford, CA

Abstract: The mammalian hippocampus is crucial for episodic memory formation, but not all of life's experiences induce equally strong memories. Notably, memories of rewarding experiences generally persist longer than memories of neutral events. Prior research has implicated both hippocampal synaptic plasticity and the activity of hippocampal place cells in the encoding of long-term episodic and spatial memories, and suggest that these processes are modulated by the presence of reward. However, due to the limitations of *in vivo* electrophysiological recording methods, it has remained unknown how hippocampal ensemble representations of rewarding experiences evolve over the course of learning and how reward may promote the long-term stability of hippocampal memory codes. To examine these issues, we capitalized on recent technical advances to conduct *in vivo* calcium-imaging studies in freely behaving mice. We expressed the genetically encoded calcium-indicator GCaMP6m and imaged the dynamics of large ensembles of CA1 hippocampal pyramidal neurons using a miniature fluorescence microscope [Ghosh et al. Nature Methods (2011) 8:871-8] and established methods for chronic time-lapse fluorescence microendoscopy [Ziv et al., Nature Neuroscience (2013) 16:264-6]. We imaged the somatic calcium dynamics of hundreds of CA1 pyramidal cells over nine days as mice learned to perform a reward-motivated spatial task. We found that as mice learned the association between place and reward there was a progressive refinement of hippocampal cells' place fields that gradually resulted in a greater preponderance of place fields at rewarded locations. Further, when we altered the spatial profile of reward contingencies, there was a rapid shift of the hippocampal representation of space that reflected the newly salient location. We expect that the detailed manner in which individual and ensemble place cell representations evolve over time will place useful constraints on models of hippocampal computation.

Disclosures: **M. Carr Larkin:** None. **E.O. Hamel:** None. **L.J. Kitch:** None. **J. Li:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mark J. Schnitzer is a co-founder of and scientific consultant to Inscopix Inc., the company that manufacturers the integrated microscope..

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: DARPA N66001-09-C-2081

DARPA N66001-09-C-2080

Title: Neural response in multiple hippocampal sub-regions to the manipulation of spatial cues in rats

Authors: *H. XU, M.-C. HSIAO, D. SONG, T. W. BERGER
USC, Los Angeles, CA

Abstract: The hippocampus is a sub-cortical structure located in the medial temporal lobe which is associated with episodic memory and the formation of new long term memory. The input information from the entorhinal cortex are transformed into output signals by the hippocampus through three sub-regions which are connected by the tri-synaptic circuit. It is believed that the hippocampus is necessary for effective spatial learning in rodents and short-term topographical memory in human. Several groups have also reported that hippocampal neurons fire not only limited to locations but also to the combination of specific memory cues presented in the location. With a specially designed microelectrode array, which consists of 16 stainless steel wires well-arranged into three groups different in length, we were able to record neural activities from multiple sub-regions of the rat hippocampus simultaneously. We first examined the spatial distribution of firing rates of the hippocampal sub-regional units while the animal was exploring freely in a highly simplified environment. The response of place cells in multiple sub-regions to the manipulation of existing spatial cues will be further investigated to understand the mechanism of spatial information encoding in the hippocampus. New objects will also be introduced into current context to explore the impact of novel elements to the spatial information representation in the hippocampus.

Disclosures: H. Xu: None. **M. Hsiao:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Program#/Poster#: 463.22/TT82

Topic: F.02. Animal Cognition and Behavior

Title: Schemas in the hippocampus: A hierarchical Bayesian approach to hippocampal place cell mapping

Authors: *S. VENDITTO[1], L. HORSTMAN[1], P. SCHRATER[2], A. JOHNSON[1]
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Abstract: Recent findings suggest that hippocampal place cell activity encodes task variables beyond space. Using a hierarchical Bayesian approach that learns the statistical structure of a behavioral task - a task schema - in order to predict future task observations, we show how predictive representations can account for (1) the modulation of place cell activity within a place field and (2) the development of multiple corresponding place fields for a single place cell as an animal develops stereotyped behavior within a task. Place cell activity within place fields is 'overdispersed' or more variable than would be expected by chance on many tasks (Fenton and Muller, 1998; Jackson and Redish, 2007). Overdispersion can be, at least partially, explained by the dynamic use of multiple reference frames within a task. For example, place field activity on the linear track is modulated by the animal's running direction. This observation can be understood as different maps for each running direction or as a single map in which both space and running direction are critical for representing an animal's position on the map. We show how structure learning and schemas can develop 'maps' that reflect both position and running direction on the linear track and more complex maps that include spatial position, context, and rules on more complex tasks. Place cells develop multiple place fields across common trajectories within repeated tasks as a task becomes stereotyped (Singer et al., 2010). We modeled the development of stereotyped behavior using reinforcement learning and then used the observations obtained from early exploration phase of learning and the later stereotyped phase of learning to examine the best predictive representation or map of the task. During early learning, the best predictive map included spatial position and the animal's last action. During later stereotyped behavior, the best predictive map reflected the sequence of the animal's most recent actions - a trajectory. This trajectory code produces multiple place fields for a single cell when trajectories are repeated. The approach provides a common explanation for overdispersion within a single place field and the development of multiple place fields across repeated task elements for a single place cell.

Disclosures: **S. Venditto:** None. **L. Horstman:** None. **P. Schrater:** None. **A. Johnson:** None.

Poster

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NSF Graduate Research Fellowship

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Cisco Systems Stanford Graduate Fellowship

Rothschild Foundation Fellowship

Title: Large-scale, ensemble representations of space by CA1 hippocampal place cells visualized during spatial learning by fluorescence calcium-imaging in freely moving mice

Authors: *L. KITCH[1], Y. ZIV[2], M. C. LARKIN[1], E. O. HAMEL[1], M. J. SCHNITZER[1]
[1]Stanford Univ., Stanford, CA; [2]Weizman Inst., Reshovot, Israel

Abstract: The mammalian hippocampus and its neuronal representations of spatial environments are thought to be crucial for spatial learning and memory. However, it remains unclear how changes in hippocampal neural codes relate to spatial learning over time scales of multiple days. Specifically, what changes in place cell firing patterns might underlie progressive improvements in spatial navigation? To address this question, we imaged the calcium dynamics of CA1 hippocampal place cells in freely behaving mice as the animals learned to navigate a radial arm maze. To do this, we used a miniature fluorescence microscope, a chronic mouse preparation for long-term imaging of hippocampus, and the genetically encoded calcium indicator GCaMP6 targeted to CA1 pyramidal cells. This approach allows us to observe the concurrent dynamics of hundreds of individual CA1 neurons during active mouse behavior. In distinction to our prior work that used this methodology but with GCaMP3, here we found that the improved sensitivity of GCaMP6 to neural spiking placed greater stringency on the capabilities of the computational image analysis procedures used for cell sorting, particularly regarding the minimization of crosstalk in the activity traces from neighboring hippocampal pyramidal cells. To meet this new challenge imposed by GCaMP6, we developed a novel cell-sorting algorithm that combines a high fidelity of calcium-transient detection with successful removal of crosstalk. Using this

approach, we next examined changes in ensemble spatial coding as navigation performance improved over the course of five days. This analysis revealed a refinement in the ensemble representation of space; as learning progressed fewer cells were active during maze running, but a larger portion of these active cells conveyed significant spatial information. A Bayesian decoding analysis revealed that spatial reconstruction errors declined as learning advanced, suggesting that hippocampal neural representations of space gradually increase in spatial accuracy as behavioral performance improves over the course of several days. Overall, our work provides an initial longitudinal account of CA1 ensemble coding dynamics during spatial learning extending over multiple days.

Disclosures: **L. Kitch:** None. **Y. Ziv:** F. Consulting Fees (e.g., advisory boards); Inscopix, Inc.. **M.C. Larkin:** None. **E.O. Hamel:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inscopix, Inc.. F. Consulting Fees (e.g., advisory boards); Inscopix, Inc..

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Grant 099926/Z/12/Z

Title: Differential regulation of cholinergic and dopaminergic systems on hippocampal place field properties

Authors: *M. TSANOV[1], O. MAMAD[2]
[1]Trinity Col. Inst. of Neuroscience, TCD, Dublin, Ireland; [2]Trinity Col. Inst. of Neurosci., Dublin, Ireland

Abstract: We investigated here if the spatial fields of hippocampal place cells can be shaped by the activity of septal and ventral tegmental projections. We examined the change of the place field properties when efferent activation was triggered 1) in the periphery of the place field and 2) in the center of the place field. After baseline recording and identification of place fields, we we applied electrical stimulation to the inputs from medial septum or ventral tegmental area every time the animal entered unit's place field and compared the firing properties of the targeted unit with the firing properties of the units with place fields in a distal location (controls). Our

first goal was to demonstrate how septal activity and theta entrainment control the spiking of hippocampal CA1 place cells and their spatial representation. Our data show that the spatial coherence, spatial information and place field rate increase for the periphery-stimulated fields, while all these parameters decrease for the center-stimulated fields. Place field size shows no significant change for both groups. Septal theta-burst stimulation evoked initial de-synchronization of hippocampal theta cells, which down-regulated the hippocampal place cell firing and place field properties. Our second goal was to investigate the effect of the ventral tegmental area (VTA) efferents on hippocampal place fields. We found that stimulation to the medial forebrain bundle evoked reshaping of the place fields with relocation of the place field centers in about 25% of the stimulated place cells. Our experiments also addressed the question of how neurons releasing dopamine and acetylcholine control the oscillatory and spiking patterns of the neurons in the hippocampal formation. We applied septal stimulation of choline acetyltransferase (Chat)::Cre and tyrosine hydroxylase (Th)::Cre rat lines (Witten et al., 2011) with optogenetic laser stimulation after the injection of ChR2-YFP adeno-associated virus in the septal cholinergic neurons or tegmental dopaminergic neurons. Here, we show that the differential modulatory control of septal cholinergic and tegmental dopaminergic neurons over the hippcampal place field representation. The cholinergic-mediated place field modulation is mediated by the rhythmic entrainment of hippocampal neurons, while the dopaminergic effect on hippocampal place cells reflects network remapping mechanisms.

Disclosures: **M. Tsanov:** None. **O. Mamad:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: HHMI

INC

Title: Place cell response variance in an attractor network model of hippocampal ca3

Authors: ***H. N. YOUSIF**[1], T. SOLSTAD[2], T. J. SEJNOWSKI[1]
[1]CNL, Salk Inst. For Biol. Studies, La Jolla, CA; [2]Ctr. for Neural Computation, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: The hippocampus is a neural structure strongly implicated in long term episodic memory. Hippocampal anatomy can be well described by implementations of recurrent neural networks (RNNs). Built on the dynamics of systems with a simple fixed point attractor structure, these models have successfully described many aspects of memory storage and recall. In addition, hippocampal place cells also have firing fields that strongly correlate with environmental location. The class of models describing this second phenomenon have hinged on the dynamics of continuous attractor neural networks (CANNs) as a computational substrate for spatial selectivity. However, current modeling efforts have shown that the fixed point attractor structure of RNNs and the spatially invariant structure of CANNs seem computationally incompatible. We propose a stochastic spiking model in a novel architecture that remedies this problem. We combine the two network types into a 2D lattice of Hopfield modules interconnected by spatially invariant weights. This model successfully replicates the spatial selectivity of hippocampal place cells without compromising the network's memory recall capabilities. This system architecture exhibits several interesting behaviors in both the spatial and temporal domain. Individual neurons have a high degree of firing variability as has been observed experimentally. However, the long-time behavior of each cell retains reliable temporal structure. Although activation of each unit is unpredictable within single field traversals, the accumulated spike total of each unit is roughly constant. We found several distinct modes of large scale network activation. These two modes exhibit highly distinct responses at the population scale when exposed to a simulated morph sequence depending on the ratio of recurrent hippocampal drive and external entorhinal drive. We explore the wide range of transitioning behaviors at the level of single neurons and discuss some of the possible explanations with regards to different hippocampal remapping paradigms. The simulated data motivates the exploration of place cell activity across large populations at timescales of order ten milliseconds.

Disclosures: **H.N. Yousif:** None. **T.J. Sejnowski:** None. **T. Solstad:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust: 095667

Wellcome Trust: 095668

Title: Influence of visual cue contrast on hippocampal place fields

Authors: *A. B. SALEEM, K. D. HARRIS, M. CARANDINI
Univ. Col. London, London, United Kingdom

Abstract: Hippocampal place cells encode estimates of an animal's position in space. These estimates are guided by cues from the environment. How does the reliability of these cues affect hippocampal activity? One possibility is that reliability influences overall firing rate. Alternatively, reliability could influence the specificity of place cells. To distinguish between these possibilities we used a virtual reality system, where the only sensory cues to position are visual, and we modulated the reliability of these cues by changing visual contrast. We trained mice to perform a visual navigation task in a virtual corridor adorned with prominent landmarks. Water-restricted mice learned to walk through the virtual room and lick at a fixed location for a water reward. Contrast was varied between a low level (18%); a medium level (60%) that was used for the majority of training sessions; and a high level (72%). Once animals performed this task accurately (typically <2 weeks), we recorded simultaneously from populations of neurons in hippocampal area CA1 and primary visual cortex (V1), using silicon microelectrodes. As expected, animals performed a large fraction of trials correctly when landmark contrast was medium or high, but their performance dropped at low contrast. Also as expected, V1 neurons responded reliably to the landmarks, with increased responsiveness at higher contrast. In CA1, many putative pyramidal cells showed place fields specific to locations of the virtual corridor. Across the population, there was no remapping or significant change in mean firing rate between contrast conditions. However, place fields were more reliable at higher contrast, with single cell spike trains 120% more predictable from spatial location than at low contrast. We used an independent Bayes population decoder to predict the position of animal based on the activity of all simultaneously recorded CA1 neurons, and found predictions to be consistently more accurate (130 %) and confident (14 %) at high compared to low contrast. We conclude that hippocampal place cells do represent the reliability of sensory cues to the animal's position. This representation affects the specificity of place fields, and not the overall firing rate of the cells. We speculate that this change in representation reflects an encoding of positional uncertainty by the hippocampal population.

Disclosures: **A.B. Saleem:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

463. Animal Models: Spatial Learning and Place Cells

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01-MH078821

NIH Grant P50-MH58880

Title: Development of distinct encoding schemes revealed by prior experience, preplay, and NMDA receptor knock-out

Authors: *G. DRAGOI, S. TONEGAWA
Picower Inst. Learning & Memory, MIT, CAMBRIDGE, MA

Abstract: Novel hippocampal spatial representations were thought to form primarily during new experiences and be specifically replayed the following sleep/rest to enable memory consolidation. Here we found that spatial tuning and noise correlations of place cell ensembles on novel tracks were reduced in naïve animals, but showed accelerated increases in the experienced ones. In both naïve and experienced mice, novel CA1 place cell sequences exhibited similar correlations with their firing sequences during sleep/rests preceding (i.e., preplay) and following the run (i.e., replay). Genetic blockade of CA3 NMDARs activity significantly delayed the experience-dependent increase in tuning and noise correlations of place cell ensembles and resulted in the expression of more rigid temporal-spatial sequences between sleep/rest and exploratory behaviors (i.e., preplay and replay). The prior, de novo spatial experience significantly reduced the NMDAR-dependence in the encoding of subsequent experiences on contiguous or isolated novel tracks. Similarly, de novo learning of a delayed alternation task was facilitated by CA3 NMDARs and this experience accelerated subsequent learning of a related task, independent of CA3 NMDARs. These results indicate that novel spatial representations develop on the framework of preconfigured hippocampal networks (i.e., preplay) whose homeostatic adjustment is accelerated by prior experience and CA3 NMDAR-dependent plasticity. Overall, these findings reveal the existence of distinct neuronal encoding schemes in the hippocampus.

Disclosures: G. Dragoi: None. **S. Tonegawa:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: AHFMR Polaris Award

Title: Lack of correlation of immediate-early gene expression patterns in the rat hippocampus during sleep and during the subsequent exploration of a completely novel environment

Authors: *A. M. DEMCHUK, M. J. ECKERT, B. L. MCNAUGHTON
Canadian Ctr. for Behavioural Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The hippocampus is believed to minimize similarities between novel and familiar memory representations by amplifying small variations in cortical input patterns, a process referred to as pattern separation (e.g., McNaughton & Nadel, 1990). Early immediate-early gene (IEG) studies (Guzowski et al., 1999; Vazdarjanova & Guzowski, 2004) and electrophysiological data (Leutgeb et al., 2007; Leutgeb et al., 2004; Muller & Kubie, 1987) tend to support the "uniform random sample with replacement" (URSWR) model, which postulates that hippocampal neurons have a uniform probability of activation and are assigned for activation by random selection. This "default" model is challenged, however, by the observed "preplay" of place cell firing sequences (Dragoi & Tonegawa, 2011), recent IEG studies that fail to demonstrate the proportionate increase in neuron recruitment predicted to accompany multiple environments (e.g., Alme et al., 2010), and by the observation that hippocampal firing rates follow a log-normal distribution (Mizuseki & Buzsaki, 2013). Altogether, these studies propose an alternative "preselection" model for neural activation where some cells have an intrinsically higher probability than others of being active in any environment or brain state. Given such evidence against the URSWR model, the purpose of this study was to assess preplay using molecular markers of neural activity that permit analysis of co-activity patterns. Specifically, we utilized double-label Arc/Homer1a fluorescent *in situ* hybridization and automated nuclear segmentation coupled with automated mRNA foci detection to analyze extensive populations of hippocampal and cortical neurons during both home cage rest and during the subsequent exploration of a remote novel environment. The observed overlap between IEG expression patterns during rest and exploration was not significantly different from that expected by random chance in dorsal CA1, CA3 and the dorsolateral entorhinal cortex. Therefore, our data do not support the preselection hypothesis (at least not in rats). A possible explanation for previous neural ensemble recording results may be that the sleep episode may have taken place near the apparatus used for recording during wakefulness. The attractor map/path integration model for place field generation (e.g., Samsonovich & McNaughton, 1997) predicts that if the "activity

bump" is allowed to move randomly around the location where it was when the rat went to sleep, it might visit states that will subsequently be observed when the rat visits nearby locations.

Disclosures: **A.M. Demchuk:** None. **M.J. Eckert:** None. **B.L. McNaughton:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: R01HD057939

Title: Tip60 HAT action in cognitive enhancement

Authors: ***S. XU**, F. ELEFANT
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Abstract: Environmental stimuli provide neurons in the brain with instructive information that shapes synaptic connections to impact cognitive ability. As such, environmental enrichment (EE) conditions have profound beneficial effects for reinstating cognitive ability in neuropathological conditions such as Alzheimer's disease (AD). While EE benefits involve epigenetic gene control mechanisms that comprise histone acetylation, the select HATs involved remain largely unknown. We have shown that Tip60 HAT action controls activity-dependant cognition linked neuronal processes that include synaptic plasticity, axonal transport and epigenetically regulates transcriptional profiles of genes enriched for these functions. Here, we examine a role for Tip60 HAT action in mediating activity-dependant adaptations to EE. The mushroom body (MB) in the *Drosophila* brain is a superb model to study cognitive processes *in vivo* as it displays homology to human circuits corresponding to the hippocampus and as such, functions in learning and memory (L&M). Here, we show that misregulation of Tip60 in the fly MB results in memory defects in these flies. AD flies also exhibit memory impairment, consistent with their inability to learn. Remarkably, excess Tip60 rescues both L&M defects in AD flies. Morphological analysis of the MB revealed that EE induced MB axonal outgrowth while Tip60 deficient flies showed a defect in such beneficial EE axonal enhancement. These defects correlated with attenuation of the transcriptional profile of certain activity-dependent cognition linked genes induced in response to EE. Our results implicate Tip60 as a critical mediator of EE-induced benefits, and

provide insight into non-invasive behavioral and epigenetic treatments for cognitive deficits in neurological disorders.

Disclosures: S. Xu: None. **F. Elefant:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Program#/Poster#: 464.03/TT90

Topic: F.02. Animal Cognition and Behavior

Support: NIA R01 AG028488

NSF GRFP

Title: The role of Cav1.2 in dentate gyrus dependent learning

Authors: *S. JIMINEZ TEMME, G. G. MURPHY
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Abstract: L-type voltage gated calcium channels (LVGCCs) have been implicated in various forms of learning, memory, and synaptic plasticity. Within the hippocampus, the LVGCC subtype, Cav1.2 has been found to be heavily expressed in the dentate gyrus. However, despite strong levels of Cav1.2 in the dentate gyrus, the role of Cav1.2 in dentate gyrus-dependent learning remains unknown. Using transgenic mice lacking Cav1.2, we investigated the role of Cav1.2 in dentate gyrus-dependent tasks. Mice were examined using Pavlovian fear conditioning and a version of the Morris water maze in which spatial cues were degraded to study pattern separation and spatial encoding. Consistent with our previous results, deletion of Cav1.2 did not impair acquisition of fear to the conditioned context associated with the footshock. However, mice lacking Cav1.2 exhibited deficits in the ability to discrimination between a context in which they received footshock and that in which they did not, suggesting deficits in pattern separation. Similarly, when Cav1.2 knock-out mice were trained using a traditional form of the Morris water maze, knock-out mice were able to learn and recall the location of the hidden platform as well as their wildtype littermates. When the task was made more difficult by restricting the number of available spatial cues, a form of the task more dependent on the dentate gyrus, mice lacking Cav1.2 were unable to encode the location of the hidden platform. These results suggest that

deletion of Cav1.2 preferentially impacts dentate gyrus function and dentate gyrus dependent learning.

Disclosures: S. Jiminez Temme: None. **G.G. Murphy:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Title: Targeted pharmacogenetic interrogation of a fear memory network

Authors: *G. VETERE[1], A. WHEELER[1], L. RESTIVO[1], S. JOSSELYN[1,2,3,4], P. FRANKLAND[1,2,3,4]
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Abstract: Long-term memories are thought to depend upon the coordinated activation of a broad network of cortical and subcortical brain regions, but within this distributed network some regions may play more important roles than others during consolidation. Previously, we used a global mapping approach to identify networks of brain regions activated following recall of long-term fear memories in mice (Wheeler et al [2013] PLoS Comp Biol). Expression analysis of the activity-regulated gene, c-fos, across 84 brain regions allowed us to identify regions that were co-active following memory recall, and presumably form a network that is engaged by long-term memory recall. Graph theoretical analysis of this network indicated that the memory network had small-world properties, and included several highly-connected hub-like regions that may play privileged roles in memory expression. Using pharmacogenetic neuronal silencing strategies, here we test the hypothesis that these hub regions play disproportionately important roles in the consolidation of long-term contextual fear memories. To do this we virally expressed the inhibitory designer receptor exclusively activated by designer drugs (DREADD) HM4Di in different hub and non-hub regions in the memory network. DREADDs are insensitive to endogenous ligands but activated by a synthetic ligand clozapine-N-oxide (CNO). When bound to CNO, this Gi-coupled DREADD induces membrane hyperpolarization and inhibition of spiking activity. Following contextual fear conditioning training, CNO or vehicle was administered via drinking water for 14 days and then contextual fear memory was tested. We

found that inhibition of several cortical and subcortical hub regions (e.g., prelimbic cortex, primary and secondary somatosensory cortex, reuniens thalamic nucleus, posterior thalamic nuclear group, laterodorsal thalamic nucleus, lateral septal nucleus, CA1 field of the hippocampus) disrupted consolidation of the contextual fear memory. In contrast, our preliminary data indicate that similar inhibition of non-hub regions in the memory network (e.g., medial geniculate nucleus) had no effect. These data support the idea that highly-connected hub regions play a disproportionately important role in the consolidation of contextual fear memories.

Disclosures: G. Vetere: None. **A. Wheeler:** None. **L. Restivo:** None. **S. Josselyn:** None. **P. Frankland:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: CONACYT (Grant 128259)

PAPIIT (Grant 201712)

Title: Memory retrieval of inhibitory avoidance induces gene expression of arc and zif268

Authors: *S. GONZALEZ[1], A. MEDINA[1], E. ALVARADO-ORTIZ[1], A. ANTARAMIAN[2], G. QUIRARTE[2], R. PRADO-ALCALA[2]
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Abstract: Gene expression is induced after retrieval of contextual and cue fear classical conditioning. However, other widely used tasks that involve multiple associations, such as inhibitory avoidance, have not been studied from the genomic perspective. In this task the animal associates the context with a foot-shock, and also undergoes an instrumental learning, as it first needs to enter to a specific place to receive the aversive consequence. We studied the expression of the genes chrm1, erk1, arc and zif268 that code for the cholinergic muscarinic receptor M1, a MAPK, a cytoskeleton protein and a transcription factor, respectively. Using Real Time PCR we measured gene expression in striatum, amygdala and hippocampus at 30, 90, and 180 min after

memory retrieval. We evaluated an Intact group of animals that remained in their home-cage, an Exploration group in which animals were trained without foot-shock, and a Trained group that underwent standard training with 1.0 mA. We found that the expression of arc was increased in hippocampus and striatum at 30 min related to memory retrieval. Zif268 changed in all the three studied regions at 30 min associated to memory reactivation; the expression in hippocampus remained increased during the three time intervals. No significant changes were observed in chrm1 and erk1 in any of the structures. The expression of arc in striatum could be important for integrating information about the current consequences of actions while the changes in hippocampus might be related to updating the information about the context. The activity of zif268 could be involved in modifying the initial information formed in striatum, amygdala, and hippocampus to readapt the behavior for future challenges. The authors thank the excellent technical assistance of Norma Serafin, Martin Garcia, Adriana Gonzalez, and Angel Méndez.

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Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR (MOP 53761)

Title: Exchange protein activated by cAMP (Epac) facilitates short-term and long-term memory in rat pup odor preference learning

Authors: ***J. H. MCLEAN**[1], M. T. GRIMES[2], M. F. POWELL[3], S. MOHAMMED[4], C. W. HARLEY[3]
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Abstract: We have been exploring how intracellular pathways interact to facilitate learning. The role of cyclic adenosine monophosphate (cAMP) leading to protein kinase A (PKA) activation and subsequent phosphorylation of cAMP response element binding protein (pCREB) has been well-characterized in vertebrates and invertebrates as an important pathway in learning. It also plays a role in early odor preference learning. Recently, an alternative signal that bypasses PKA

has been found to affect long-term potentiation and memory in adult rodents. This is exchange protein activated by cAMP or Epac. Here we test the hypothesis that Epac is involved in short- or long-term memory formation in early olfactory learning in parallel with the cAMP/PKA pathway. We used an agonist to Epac (8-pCPT) to investigate the role of Epac in neonate rat learning. We found that when 8-pCPT was infused into the olfactory bulb before odor presentation, the Epac agonist acted as an unconditioned stimulus to induce both 3h and 24h memory. By pairing Epac with a different odor than tested, we also determined that the effect of the agonist was specific to the paired odor and did not generalize to an unpaired odor. Western blot analysis showed that the Epac agonist induced ERK phosphorylation 10 min after training, however, the effect on pCREB was equivocal. The effect of 8-pCPT on pERK activation was confirmed by immunohistochemistry which showed strong pERK label in the dendritic field of mitral cells in the olfactory bulb when locally infused and paired with odor while pCREB expression itself was only moderately changed. Thus, the mechanism by which Epac acts in the olfactory bulb as an associative input appears to be via phosphorylation of ERK. These data add a parallel pathway supporting odor preference learning and memory that would also normally be recruited with an increase in olfactory bulb noradrenaline. How the two cAMP-activated pathways interact in this model is not yet determined, but prior investigations suggest the PKA pathway is not involved in short-term memory support. The Epac pathway is the first to be causally related to short-term memory in early odor preference learning.

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Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Title: GSK3β inhibition in the hippocampus and striatum is task-specific

Authors: *C. J. SCAVUZZO[1], P. E. GOLD[2], D. L. KOROL[2]
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Abstract: GSK3β is a constitutively active protein kinase involved in fundamental cascades important, for example, for glucose metabolism, inflammation, and apoptosis. The hyperactivity

of GSK3β during neurodegeneration has been heavily studied in transgenic mice and animal models of disease. For example, GSK3β inhibition prevents degeneration and attenuates learning deficits in mouse models of Alzheimer's disease. Recent interest in GSK3β has revealed that inhibition via serine-9 phosphorylation occurs following neural stimulation and after treatment with trophic factors such as BDNF. Surprisingly, very little is known about the role of GSK3β in learning in healthy young adult rats. We hypothesized that engaging in learning tasks that increase BDNF levels would increase GSK3β inhibition. Previously, we found that priming with spontaneous alternation (SA) testing enhanced place and response learning and increased BDNF content in the hippocampus and striatum. We also found that extracellular concentrations of BDNF increase in the hippocampus and striatum during place and response learning, respectively. To determine if tasks that increase BDNF also increase GSK3β inhibition, six groups of rats were tested: 1) untested control rats; 2) rats tested on SA only; 3) rats trained only on place learning; 4) rats trained only on response learning; 5) rats primed with SA prior to place learning; 6) rats primed with SA prior to response learning. Rats were sacrificed immediately after training, the hippocampus and striatum were harvested and processed for PAGE, and pGSK3B and total GSK3B were quantified via Western blot analysis. GSK3β inhibition was determined by within-rat ratios of phosphorylated to total GSK3β from each structure. Training on the place and response tasks robustly increased GSK3β inhibition in the hippocampus and striatum, respectively, compared to untested controls and to rats tested on SA alone. Increases were not as reliable in non-canonical structures. Despite enhancements in learning and BDNF content following SA priming, priming did not further increase hippocampal or striatal GSK3β inhibition in rats trained on place or response tasks. Unexpectedly, SA testing itself did not increase GSK3β inhibition in the hippocampus or striatum compared to untested controls. However, SA testing is qualitatively and quantitatively different from the other tasks: SA testing has no food reward, may have a lower cognitive demand, and lasts for only 20 min instead of 1 hr. Thus, our data suggest that learning-induced GSK3β inhibition in specific brain regions may dissociate by task and may require a sufficient level of cognitive demand or arousal.

Disclosures: **C.J. Scavuzzo:** None. **P.E. Gold:** None. **D.L. Korol:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH IRP

Title: A proteomic approach to elucidate the molecular signaling pathways underlying visual recognition memory in monkeys

Authors: *B. A. CORGIAT[1,2,3], C. MUELLER[2], J. N. TURCHI[3], J. L. OLDS[1], R. C. SAUNDERS[3], L. A. LIOTTA[2], M. MISHKIN[3]
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Abstract: The role in visual recognition memory of the perirhinal cortex is well established, as is the role of cholinergic activation of perirhinal M1 receptors. Furthermore, neurophysiological data demonstrate that successful visual memory formation is characterized by enhanced multiunit activity in the upper middle and deep layers of perirhinal cortex. However, the M1 muscarinic-dependent intracellular signaling profiles underlying critical synaptic changes within these perirhinal laminae remains unclear. Here we present a proteomics approach to uncover the molecular signaling pathways activated during memory formation in a region-specific manner using two techniques: laser capture microdissection and reverse phase protein microarrays (RPPA). Sections of snap frozen perirhinal tissue from four Rhesus monkeys were prepared and stained for Nissl substance. Tissue subregions of perirhinal cortex (Layers III and V/VI), as well as hippocampus (cell body layers of CA1, CA3, and dentate gyrus and their corresponding dendritic fields), were isolated using laser capture microdissection. Lysed tissue samples were then printed onto RPPA. These arrays were probed with antibodies against phosphorylated, as well as total, proteins involved in muscarinic signaling, synaptic transmission, and neuronal activity. This methodology will allow us to elucidate which critical proteins have been activated in specific laminae of the perirhinal cortex during visual memory formation.

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Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: R00MH093459 to NCT

Title: Persistent dysregulation of memory and signal transduction by peripheral inflammation

Authors: *E. J. DONZIS, N. NEVÁREZ, I. C. SPEIRS, A. A. SCHMELING, L. M. TURNBULL, N. C. TRONSON
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Abstract: Chronic systemic inflammation is associated with dysregulation of memory and mood in both animal models and patients of chronic illness such as major surgery and heart attack. The mechanisms by which inflammation causes these changes remain largely unknown. Here we examined the molecular mechanisms involved in memory and emotional dysregulation using a surgically-induced myocardial infarction (MI) in both male and female mice as a peripheral inflammatory event. We have previously demonstrated that male, but not female mice exhibit enhanced context fear-related memory and depression-like behavior two weeks after an MI. In contrast, MI impairs context fear-related memory and induces depression-like behaviors in both male and female mice eight weeks post-surgery. Sham surgical treatment induced equivalent changes in females but not males. To determine the progression of the inflammatory response, we used multiplex analysis of 32 cytokines including IL-1β, IL-2, IL-4, IL-6, TNFα, and IL-10 in blood serum and hippocampus at multiple time points between 3 days and eight weeks after surgery in MI and sham-operated mice. In addition, we determined alterations of memory-related signaling cascades, including MAPK, AKT, STAT3, CEPB/β, NFκB, and CREB in the dorsal hippocampus after context fear conditioning. We observed distinct patterns of peripheral and central cytokines changes in males and females and sex-specific dysregulation of kinase signaling pathways eight weeks after surgery. These findings suggest that systemic inflammation results in long lasting alterations of signaling cascades that mediate memory impairments and depression.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NWO Grant R2259

Title: Mineralocorticoid receptors as genetic resilience factor under chronic stress?

Authors: *S. KANATSOU[1,2], A. P. HARRIS[3], J. R. SECKL[3], H. KRUGERS[2], M. JOËLS[1]
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Abstract: Stress is a critical environmental risk factor for the development of cognitive deficits and brain disorders such as depression. Susceptibility to disorders is to a large extent also determined by genetic background. For instance, the common mineralocorticoid receptor (MR) I180V variant, which results in diminished cortisol-induced MR gene expression, has been associated with geriatric depressive symptoms. The goal of the current study was to examine, in a transgenic mouse model, whether high MR expression may act as a protective factor against the effects of chronic stress. Two-months old transgenic male mice overexpressing the MR in the forebrain (MR-Tg) and control littermates were subjected to 21 days of chronic unpredictable stress (CUS). On day 22, mice were sacrificed and body weight gain as well as adrenal and thymus weight was determined. Furthermore, we examined the effects of CUS on anxiety-related behaviour using the elevated plus maze as well as learning and memory performance, using the contextual fear conditioning and object-in-context tests. In addition, we investigated the different phases of adult neurogenesis in the hippocampus using immunohistochemistry to assess cell proliferation, newborn cell survival and the number of young, differentiating neurons. CUS significantly reduced body weight gain and thymus weight and increased adrenal weight, in both MR-Tg and wildtype littermates. CUS hampered spatial learning, while MR overexpression itself did not affect spatial learning. Interestingly, the effects of CUS on spatial learning were absent in MR overexpressing mice. Both CUS and MR overexpression enhanced contextual fear conditioning, but no interaction between CUS and MR expression was found. Moreover, there was no difference between MR-Tg and control littermates in anxiety. Structural plasticity in the dentate gyrus was examined by determining the number of BrdU+, Ki67+ and DCX+ cells.. We analysed both the total number of dentate cells, and the number of cells in the supra- and infrapyramidal blades. Overall, we did not observe a main effect of treatment, genotype nor an interaction with regard to the total number of BrdU+ or Ki67+ cells. CUS did cause a main effect of treatment in the total number of DCX+ cells in the dentate and in the infrapyramidal blade. Moreover, in the latter region we observed a significant interaction effect. Post-hoc analysis revealed a significant reduction in the number of DCX+ cells after CUS in wildtypes ($p<0.005$), while CUS had no effect in the MR-Tg animals. We conclude that MR overexpression may protect against chronic stress-induced impairment of hippocampal function and- to some extent - neurogenesis.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Forebrain-specific knockdown of Staufen2 in transgenic rats impairs synaptic plasticity and learning and memory processes

Authors: S. BERGER[1], I. FERNÁNDEZ-LAMO2[2], K. SCHÖNIG[1], S. CLEMENTI[1], T. ENKEL[1], M. A. KIEBLER[3], S. GROTHE[4], O. VON BOHLEN UND HALBACH[4], J. M. DELGADO-GARCÍA[2], A. GRUART[2], *D. BARTSCH[1]
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Abstract: Dendritic localization and translation of a subset of mRNAs plays a central role in synaptic plasticity and learning and memory. The RNA-binding proteins of the Staufen (Stau) family are essential for the targeting of mRNAs in diverse cell types from *Drosophila* to mammals. In *Drosophila*, the Staufen-Pumilio pathway is crucial for long-term memory. In mammals, two genes Stau1 and Stau2 are homologues of *Drosophila* Stau proteins. In contrast to the more ubiquitously expressed Stau1, Stau2 is mainly expressed in the nervous system. Stau2 is incorporated into messenger ribonucleoprotein particles (mRNPs) that are transported along microtubules in dendrites. In cell culture experiments, Stau2 was shown to be involved in the formation of dendritic spines and associated with changes in their morphology and in synaptic efficacy. To delineate the role of Stau2 in synaptic plasticity and learning and memory in the mammalian brain *in vivo*, we generated a transgenic rat model, in which Stau2 has been conditionally inactivated by inducible miRNA expression in principal neurons of the forebrain. This rat model is composed of two separate transgenes: i) an inducible cre recombinase (CreERT2), expressed via the forebrain-specific αCaMKII promoter and ii) a miRNA targeting Stau2 mRNA, coexpressed with eGFP through the ubiquitous CAGGS promoter. Forebrain-specific expression of the miRNA is initiated only after removal of a floxed “STOP” cassette by the application of tamoxifen in adult animals and the resulting activation of the Cre recombinase. Tamoxifen-induced double transgenic animals (stau2KD) show a high degree of eGFP-positive cells in hippocampal regions CA1 and CA3 and in cerebral cortex. Immunohistochemical analysis of stau2KD rat brains demonstrated knockdown of Stau2 protein in eGFP-positive

neurons. While there are no major anatomical defects, hippocampal dendritic spines are reduced both in size and number. *In vivo* electrophysiological recordings carried out in parallel in CA1, CA3 and DG regions of the hippocampus in freely moving animals indicate that Stau2 protein plays an important role in the balance between LTP and LTD during the learning task. In addition, Stau2 knockdown produced a significant learning deficit and changes in activity-dependant strength of hippocampal synapses during task performance. Stau2 knockdown also leads to impairment in novelty recognition and deficit in spatial working memory. In conclusion, our results suggest that Stau2 protein is critically involved in synaptic plasticity and changes in Stau2 dependent synaptic plasticity translate into altered learning and memory in Stau2 deficient animals.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Mitochondrial cannabinoid receptor mediate side-effects of cannabinoids

Authors: *T. C. DESPREZ[1,2], E. HEBERT-CHATELAIN[3], E. SORIA-GOMEZ[2], L. BELLOCCHIO[4], A. DELAMARRE[2], A. BUSQUETS-GARCIA[2], L. ROBIN[2], G. TERRAL[2], N. PUENTE[5], P. GRANDES[5], F. MASSA[2], G. BÉNARD[2], G. MARSICANO[2]
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Abstract: Cannabinoids exert potential therapeutic effects (e.g. analgesia), accompanied by important side effects (e.g. catalepsy and amnesia), but the specific molecular mechanisms involved are poorly understood. The lipid cell-penetrant antagonist AM251 injected into the substantia nigra reticulata (SNr) blocked cannabinoid-induced analgesic and cataleptic effects. However, a peptide non-cell penetrant antagonist (hemopressin) blocked only analgesic effects, suggesting the participation of intracellular CB1 receptors in sedative effects. We found that

intracellular mitochondrial CB1 receptors (mtCB1) regulate brain bioenergetic processes via inhibition of the mitochondrial soluble adenylyl cyclase (sAC). Interestingly, intra-SNr injections of the sAC inhibitor KH7 blocked the cataleptic, but not the analgesic effect of cannabinoids. Additionally, we generated a mutant CB1 protein (DN22-CB1) lacking mitochondrial localization. Cannabinoid-induced memory impairment was rescued by viral hippocampal re-expression of wild-type CB1 in CB1-KO mice, but not of DN22-CB1. Thus, cannabinoids exert important side effects (catalepsy and memory impairment) likely via activation of mtCB1 receptors.

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Poster

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NARSAD 21069

Title: Dissecting differential roles for Akt isoforms in signaling, synaptic plasticity, and behavior

Authors: *J. LEVENGA[1,3], M. ROCHE[1,3], H. WONG[1,4], C. HOEFFER[2,3]
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Abstract: Akt serine/threonine kinases play a key role in neuronal morphogenesis, cell growth, metabolism and survival. Emerging evidence has implicated the involvement of impaired Akt signaling in human psychiatric disorders including schizophrenia and depression. There are three different isoforms of Akt (Akt1, Akt2 and Akt3) and all are expressed in the brain. We took

advantage of knockout and floxxed strains for single Akt genes to investigate the role of individual isoforms in the brain. We examined behavior, synaptic plasticity and biochemical signaling in wild-type and knockout Akt-mutant mice. First, we conducted a behavioral battery to examine general well-being of the mutant mice in the open field arena (OFA) and elevated plus maze (EPM). Then the mice were tested for social approach, novel object recognition (NOR) and associative fear conditioning followed by extinction training and testing. Next, we studied hippocampal synaptic plasticity in Akt mutant mice. Because it was previously shown that tetanic stimulation and stimulation of the group I mGluR receptors induce Akt phosphorylation leading to increased kinase activity, we hypothesized that Akt isoforms may play differential roles in synaptic plasticity. In addition to genetic blockade of isoform-specific Akt signaling, we also used the pan-Akt inhibitor MK-2206 to examine the effect of pharmacological blockade of Akt activity on synaptic plasticity. Finally, we examined the expression and localization of each Akt isoform in the brain using immunohistology. Our results show different localization of Akt isoforms, suggesting the requirement of each isoform in cell-specific signaling for optimal performance in the adult mouse brain. In conclusion, isoform-specific mechanisms may define a specific and targetable involvement in neuronal signaling for Akt kinases in human neurological diseases and disorders.

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Poster

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Support: NASA Grant NNX13AB66G

Title: Long-term effects of space radiation-induced ablation of neural progenitor cells on hippocampal synaptic plasticity, learning and memory

Authors: *O. MIRY[1], K. R. GOPAUL[2], X.-L. ZHANG[2], J. A. MONCASTER[3], C. E. TAGGE[3], L. E. GOLDSTEIN[3], P. K. STANTON[2]
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Abstract: The dentate gyrus subgranular zone (SGZ) is one of two brain regions undergoing neurogenesis throughout adult life. The functional significance of granule cell turnover, especially in sustaining learning and memory, is poorly understood. Low doses of high energy particle radiation (LDRp), experienced by astronauts on NASA missions such as SkyLab and the International Space Station, reduces hippocampal neurogenesis in adult mice. We utilized this model of progenitor cell-ablation to determine whether learning and memory are persistently impaired in the absence of steady neurogenesis in the SGZ. Young-adult mice received whole-body particle radiation from a 28Si source (100 centiGray) using the particle beam line facilities at the National Space Radiation Laboratory of Brookhaven National Laboratory, and were compared to off-axis sham irradiation controls. Chronic effects of LDRp, 20 months post-exposure, on synaptic plasticity were assessed following chemically-induced (Forskolin + Rolipram, 10 μM each) cyclic AMP-dependent, or theta-burst stimulus-induced long-term potentiation (LTP) of Schaffer collateral-CA1 synapses in hippocampal slices. LDRp produced a marked enhancement in the magnitude of stimulus-evoked LTP and chemically-evoked cAMP-dependent LTP ($P < 0.05$, repeated-measures ANOVA). Consistent with these data, separate cohorts of LDRp-exposed mice also scored better in memory acquisition in a Barnes maze hippocampus-dependent spatial memory task ($P < 0.05$, repeated-measures ANOVA), and performed slightly better on memory retention, than sham controls. In contrast, LDRp-exposed mice were no different from sham controls in locomotion or exploratory activity in open-field or Elevated Plus maze testing. While LDRp has been shown to reduce neurogenesis in the hippocampus, no studies have examined the long-term effects at such a late post-exposure time point. Enhanced learning acquisition and activity-dependent long-term synaptic plasticity suggests that compensatory changes in existing neurons may result in a lower threshold for induction of LTP. Further experiments will characterize the time course and regional specificity of the effects of LDRp on synaptic plasticity and cognition.

Disclosures: **O. Miry:** None. **K.R. Gopaul:** None. **X. Zhang:** None. **J.A. Moncaster:** None. **C.E. Tagge:** None. **L.E. Goldstein:** None. **P.K. Stanton:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 464.15/UU10

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant

Dept. of Psychiatry Dalhousie University

Title: Maternal care effects on ATRX expression, genome stability and long-term neurobehavioral development

Authors: *A. KORGAN[1], A. HUNDERT[2], I. C. G. WEAVER[1,3]
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Abstract: Mounting evidence indicates that the maintenance of chromatin architecture is essential for normal human development and cognitive function. The ATRX gene, which is essential for normal growth and cognitive development, encodes a chromatin-remodeling protein that is expressed in developing neural structures, including newly-born cortical and hippocampal neurons in mice. Employing a new model of gestational psychological stress, in combination with well-established models of physical prenatal stress, we have shown that maternal behavior mediates the effects of gestational psychological stress on neural ATRX gene expression in the offspring, which is associated with alterations in DNA methylation, DNA damage signaling and stable individual differences in learning and memory and anxiety-related and social behavior, as well as cortical and hippocampal function in the adult animal. These results and those from cross-fostering studies support the possible involvement of ATRX in the sensitization of neurons to stress hormones and the programming of somatic behavior in response to maternal care. Since disruption of ATRX impairs cognition and motor functions, inhibits learning in mouse pups, and contributes to developmental silencing of imprinted genes that shape somatic growth and brain, we hypothesize that the effects of mother-offspring interactions during the first week of postnatal life on ATRX expression and function influences both genome integrity and gene expression programs that underlie normal cognitive and emotional development. The elucidation of the mechanisms involved in the impact of neonatal nurturing addresses perhaps the most challenging question in development: How are experiences occurring in early life rendered permanent? In the case of genome stability and sustained changes in gene expression in brain cells, we can begin to understand the neurobiological basis for individual differences in personality and cognition as well as related risk for learning and behavioural disorders (e.g., Autism Spectrum, ADHD).

Disclosures: A. Korgan: None. **A. Hundert:** None. **I.C.G. Weaver:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: NRF-2013R1A1A1006766

NRF-2013R1A3A1072570

Brain Korea 21 Plus

Title: Effects of cell type-specific expression of a mutant SHP-2 on learning and memory

Authors: H. RYU[1], T. KIM[2], M. KANG[1], B.-K. KAANG[2], *Y.-S. LEE[1]
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Abstract: RAS-MAPK signaling cascade plays critical roles in various functions in the nervous system including synaptic plasticity and learning and memory. The misregulations of RAS-MAPK signaling are associated with many neurodevelopmental disorders such as neurofibromatosis type I (NF1) and Noonan syndrome (NS). Previous studies suggested that the imbalance between excitatory and inhibitory neurotransmission might be the cellular mechanism underlying learning and memory deficits in the mouse models of several neurodevelopmental disorders including NF1. Here, we overexpressed a NS-associated mutant SHP-2 that upregulates Ras signaling in different neuronal types to determine whether the learning and memory deficits in NS are caused by changes in the signaling in either excitatory or inhibitory neurons. We infused floxed AAV vector into the hippocampi of cell type-specific CRE mouse lines and examined the behavioral and electrophysiological phenotypes. This study will provide a critical insight for the understanding and developing treatments for cognitive deficits in NS.

Disclosures: H. Ryu: None. **T. Kim:** None. **B. Kaang:** None. **Y. Lee:** None. **M. Kang:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Program#/Poster#: 464.17/UU12

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Title: The beneficial role of the bone derived hormone osteocalcin (OCN) in cognitive functions

Authors: *S. KOSMIDIS[1], L. KHRIMIAN[2], F. OURY[3], G. KARSENTY[3], E. R. KANDEL[4]
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Abstract: We have recently provided evidence that age related memory loss is a distinct entity different from Alzheimers's disease. It differs in age of onset, area of onset, and molecular mechanism (Pavlopoulos et al. 2013). We also provided evidence that one of the molecules responsible for age related memory loss is the Histone binding protein RbAp48. RbAp48 exerts its effects by regulating histone Acetylation. Recently, Karsenty and his colleagues found that the mouse bone-derived hormone osteocalcin (OCN), is necessary for several cognitive functions such as anxiety and depression, and possibly memory storage. We here find that systemic and acute delivery of OCN in young and aged wild type mice improves cognitive functions of young mice and reverses aged related memory loss. These effects are mediated in part by the activation of the PKA-Creb signaling cascade and RbAp48.

Disclosures: S. Kosmidis: None. **L. Khrimian:** None. **F. Oury:** None. **G. Karsenty:** None. **E.R. Kandel:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC DG

OMHF

Title: Age-related changes in hippocampal Arc expression following minimal behavioral induction

Authors: *I. V. ODINTSOVA[1], B. J. SCHMIDT[2], E. J. MARKUS[3], D. F. MARRONE[1,4]
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Abstract: Normal aging is associated with significant changes in cognitive function, including memory decline. The neurobiological underpinnings of age-related memory deficits include aberrant changes in gene products that mediate plasticity in the aged brain. Many studies,

however, show that differences in plasticity are greatest when stimulation is near the threshold for plasticity induction, and it is this peri-threshold plasticity that is of the greatest relevance for age-related changes in memory function. To test this hypothesis, we examined the expression of the immediate-early gene Arc (a gene product critical for lasting memory and enduring plasticity) in adult (11 months) and aged (23 months) F344 rats as they traverse a varying number of laps in a triangular track (i.e., 1, 3, or 5). These limited numbers of passes through hippocampal place fields provide a behavioral approximation to peri-threshold plasticity induction. Using fluorescence *in situ* hybridization (FISH), we identified the proportion of pyramidal neurons that express Arc following these treatments. Adult and aged rats expressed Arc in comparable numbers of cells throughout all hippocampal regions under all conditions. Further examination will determine if the amount of Arc produced (and thus the intensity of fluorescence in individual cells) will vary with age under these minimal stimulation conditions. Any differences found may contribute to the memory deficits associated with advancing age.

Disclosures: I.V. Odintsova: None. **B.J. Schmidt:** None. **E.J. Markus:** None. **D.F. Marrone:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH087463

NIH Grant S10RR027374

Title: Object-location training elicits an overlapping but temporally distinct transcriptional profile from contextual fear conditioning

Authors: *S. G. POPLAWSKI[1], H. SCHOCH[1], M. WIMMER[1], J. D. HAWK[2], T. ABEL[1]
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Abstract: Hippocampus-dependent learning is known to induce changes in gene expression, but information on gene expression differences between different learning paradigms that require the hippocampus is limited. The bulk of studies investigating RNA expression after learning use the contextual fear conditioning task, which couples a novel environment with a noxious footshock.

Although contextual fear conditioning has been useful in discovering gene targets, some of the genes induced during this training paradigm may be a response to the stress of the footshock. In this study, we used the object-location memory task, which does not require a noxious stimulus, and studied gene expression at two time points after learning in a high-throughput manner using a microfluidic qPCR approach. This technology allows for 9,216 simultaneous qPCR reactions, facilitating highly complex gene expression experiments in a fraction of the time. We found that expression of the classic immediate-early genes change after object-location training in a fashion similar to that observed after contextual fear conditioning. However, the temporal dynamics of gene expression are different between the two tasks, with object-location memory producing gene expression changes that last at least 2 hours. Our findings indicate that different training paradigms may give rise to distinct temporal dynamics of gene expression after learning.

Disclosures: S.G. Poplawski: None. **H. Schoch:** None. **M. Wimmer:** None. **J.D. Hawk:** None. **T. Abel:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Support: NIH Training Grant T32-MH017168

NIH Grant R01-MH087463

NRSA Fellowship 1F31NS079019

Title: Regulation of hippocampal synaptic plasticity and memory by the co-repressor Sin3a through Homer1/mGluR5 signaling

Authors: *M. BRIDI[1], H. SCHOCH[2], C. FLORIAN[3,5], S. G. POPLAWSKI[4], J. D. HAWK[1,6], R. HAVEKES[3], T. ABEL[3]
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Abstract: Long-term memory consolidation and long-term potentiation depend on the control of activity-dependent neuronal gene expression, and the expression of many plasticity-related genes

is regulated by epigenetic mechanisms such as the post-translational modification of histones. Histone deacetylase (HDAC) enzymes have been identified as negative regulators of hippocampus-dependent memory and synaptic plasticity, and pharmacological inhibition of HDACs has been shown to enhance long-term potentiation and several forms of long-term memory in rodents. HDACs and other histone-modifying enzymes are recruited to their regulatory targets as members of large co-repressor complexes, but the role of these co-repressors in neuronal function is poorly understood. Sin3a is one of these transcriptional co-repressor proteins; it coordinates a core complex that includes the Class I histone deacetylase enzymes HDAC1 and HDAC2, and also interacts with repressive factors that have been implicated in memory including MEF2, MECP2, NCoR, and REST. To investigate the role of the co-repressor Sin3a in memory and plasticity, we used the Cre-LoxP system to conditionally delete a portion of Sin3a in excitatory forebrain neurons. Here, we show that mutation of Sin3a enhances long-term potentiation (LTP) in hippocampal slices, and also occludes LTP enhancement by administration of the HDAC inhibitor Trichostatin A. Sin3a mutants also exhibit enhanced long-term (but not short-term) contextual fear memory, and following fear conditioning show increased expression of the kinase Cdk5 and the synaptic scaffold Homer1, as well as increased phosphorylation of ERK1/2. Sin3a mutants also exhibit long-lasting LTP that requires the Group I metabotropic glutamate receptor mGluR5, but is independent of mGluR1α. Our studies indicate a role for Sin3a as a negative regulator of memory of synaptic plasticity, acting through Homer1/mGluR5 signaling.

Disclosures: **M. Bridi:** None. **H. Schoch:** None. **S.G. Poplawski:** None. **T. Abel:** None. **R. Havekes:** None. **J.D. Hawk:** None. **C. Florian:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: Irish Research Council for Science, Engineering and Technology

Title: Regulation of the transcription factor REST/NRSF and putative target genes during memory consolidation and following glutamate stimulus

Authors: ***D. C. CORBETT**, K. J. MURPHY
Univ. Col. Dublin, Dublin, Ireland

Abstract: Learning requires glutamate-mediated hippocampal synaptic plasticity underpinned by a cascade of de novo gene transcription and protein synthesis. Previously in our laboratory a microarray study characterised the transcriptional profile in the first 24h of memory consolidation. Bioinformatics analysis identified several potential transcription factors (TF) involved in learning, including REST, a master repressor of neuronal genes which has roles in neuronal development and synaptic plasticity. Here we used immunofluorescence and confocal imaging to examine REST expression and localisation in the adult rat hippocampus at basal conditions and following passive avoidance (PA) learning. We found that REST is endogenously expressed, with higher levels in the pyramidal neurons than in the granular neurons. After PA, animals showed learning-associated REST regulation that was time and region-specific. At 1h following PA, nuclear REST protein was up-regulated in the three main regions of the hippocampus, dentate gyrus (DG) (51±11% vs passive control; $p<0.001$, Student's t-test), CA1 (30±18%; $p<0.001$) and CA3 (50±15%; $p<0.01$). At 2h and 3h, REST was down-regulated in the DG (36±3% and 14±2%, respectively; $p<0.001$) and CA1 (30±7% and 26±4%, respectively; $p<0.001$). Using qPCR, we observed down-regulation of Rest mRNA at 2h in the CA region. To further characterise the relevance of REST regulation following learning, we cross-referenced our microarray list of learning-associated genes with a list of REST target genes and identified 88 putative learning-associated REST targets, mainly regulated at 2-6h post-learning. Functional analysis of these genes revealed that 1-3h, genes pertaining to structural synaptic remodelling (e.g. Slitrk1) and neurotransmission (e.g. Gabbr1) categories were prevalent. Using qPCR, we found that both Slitrk1 and Gabbr1 genes were down-regulated at 2h following PA, and Slitrk1 was up-regulated at 3h, agreeing with REST regulation at protein level. Lastly, we investigated whether memory-associated REST regulation could be driven by physiological increases in glutamate. In primary hippocampal neurons, REST protein was increased at 1-3h post-glutamate treatment, and at 3h, REST regulation was NMDA receptor-dependent. In conclusion, we propose a novel role for REST in memory consolidation as a regulator of learning-associated genes, possibly driven by glutamate neurotransmission via the NMDA receptor.

Disclosures: D.C. Corbett: None. **K.J. Murphy:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: RFBR-13-04-40334-H

Title: Reverse-transcriptase inhibitor 3'-azido-3'-deoxythymidine impairs hippocampal-dependent memory in mice

Authors: ***M. ROSHCHINA**[1], O. IVASHKINA[1], D. ZUBKOV[2], K. ANOKHIN[1,3,4,5]
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Abstract: Reverse-transcription mediated retrotranspositions of L1 mobile elements was demonstrated to occur in the adult brain in environment-dependent manner, though functional significance of this process remains unclear. In the present study we tested whether enriched environment and intensive learning (EE-IL model) can activate L1 retrotranspositions in the brain. Mice from EE-IL group were housed in cages with the enriched environment and then were sequentially trained in the spatial Morris water maze (MWM), fear conditioning and active avoidance tasks. Number of L1 DNA copies in hippocampus measured by qPCR was increased in the EE-IL group compared to control mice. Next we examined whether the selective inhibitor of reverse transcription 3'-azido-3'-deoxythymidine (AZT) can affect long-term memory consolidation in various learning tasks: step-down passive avoidance (SDPA), cued and contextual fear conditioning (CuedFC and ConFC), spatial and non-spatial MWM and taste aversion (TA). Administration of AZT (30 mg/kg, i.p., 30 min before training) produced amnesia in SDPA, CuedFC, ConFC and in spatial MWM, but not in the non-spatial MWM or TA. AZT administration did not alter mouse behavior in the elevated-plus maze and open field tests. Also we examined the dynamic of AZT-induced amnesia in ConFC. Injection of AZT (30 mg/kg, i.p.) 30 min before fear conditioning produced amnesia in contextual test 3, 7 and 45 days later but had no effect on contextual memory in tests 6 or 24 h after training. Thus our experiments demonstrate that EE-IL experience can increased copy number of L1 elements in the adult mouse brain genome. Also our experiments demonstrate that nucleoside analog reverse-transcriptase inhibitor AZT produces strong impairment of long-term hippocampus-dependent forms of memory. Altogether these data suggest that formation of long-term memory in certain tasks can depend on the reverse transcription, the possible source of which is experience-dependent activation of retrotransposons in the adult brain cells.

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Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Title: Effects of stimulation and blockade of 5-HT6 receptor during the formation of memory and amnesia

Authors: *F. APARICIO NAVA[1], A. MENESES[1], G. LIY-SALMERON[2]
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Abstract: Evidence suggests that the use of agonists and antagonists of the 5-HT6 receptor improve memory and reverse the amnesic effect, however the pharmacological and molecular mechanisms involved are unknown. Autoshaping test is a self-learning method useful for evaluating promnesic or antiamnesic effects: Hence, 5-HT6 receptor agonists and antagonists were tested during memory formation and amnesia models (scopolamine 0.17 mg; muscarinic antagonist cholinergic, and dizocilpine 0.2 mg; glutamatergic antagonist). Animals were given autoshaping training, and post- training treated (IP, SC; PO) (control mg/kg), scopolamine; .17), dizocilpine (.2), 5-HT6 receptor agonist EMD (1), the 5-HT6 receptor antagonist SB-399885 (10), SB-399885-scopolamine, SB-399885-dizocilpine, EMD-scopolamine, EMD-dizocilpine, SB-399885-EMD- scopolamine, SB-399885-EMD-dizocilpine. Memory was evaluated 1.5 hrs (short-term memory, STM) and 24 hrs (long-term memory, LTM) later. STM was impaired by scopolamine or dizocilpine, without affecting LTM. EMD or SB-399885 alone had no effect on STM and SB-399885 improved LTM. EMD or SB-399885 reversed the STM decrement induced by scopolamine or dizocilpine. The scopolamine-SB-399885-EMD or dizocilpine-SB-399885-EMD combination produced normal STM and LTM. In conclusion, 5-HT6 receptor agonist and antagonist reversed memory impairment. These are being used for western blot analysis for neural transporters.

Disclosures: F. Aparicio Nava: None. **A. Meneses:** None. **G. Liy-Salmeron:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Title: Single-neuron RNA-seq: Genomic dissection of memory circuits and cell census in the brain

Authors: *L. L. MOROZ[1,2], G. MEREDITH[5], Y. SUN[5], K. M. CANDELARIO[3], D. DHINGRA[5], L. IANOV[4], A. RANI[4], S. HARDEN[2], A. KUMAR[4], C. J. FRAZIER[2], D. A. STEINDLER[3], T. FOSTER[4], A. KOHN[1]
[1]The Whitney laboratory for Marine Biosci., Univ. of Florida, Saint Augustine, FL; [3]Neurosurg., [4]Neurosci., [2]McKnight Brain Inst., Gainesville, FL; [5]Ion Torrrent, ThermoFisher, San Francisco, CA

Abstract: The human brain is the most complex structure composed of ~86 billion neurons - with most having a unique molecular signature. Here, we developed a fast and cost-efficient method for parallel capture and single-neuron transcriptome profiling using an array of nanofluidic integrated circuits followed by high-throughput semiconductor sequencing. Both human neuroprecursor cells, rodent hippocampal neurons and individual neurons from *Aplysia* memory-forming circuit were used to validate the methodology, which is not limited by the size of the neurons (7-30 um). The protocol allows performing 400-600 transcriptomes per run significantly reducing the cost of the single-cell RNA-seq. As the result, we reliably identify and quantify virtually all neuronal RNAs and revealed enormous variability ($0.69<r<0.84$) in the transcriptional output among morphologically similar cell types. From 7,710 to 17,042 of protein-coding genes were captured in each cell. A large fraction of obtained sequencing reads correspond to intergenic, intronic and UTR regions implying that a large portion of the human genome is transcribed even in a single neuron. In the human neuroprecursor cells, one of the most abundantly expressed transcripts is the long noncoding RNA called metastasis associated lung adenocarcinoma (MALAT1) involved in regulating alternative splicing and implicated in

various cancers. MALAT1 was differentially expressed across all the single cells tested. Other differentially expressed transcripts were the lncRNA ANP32E, and the SOX family of transcription factors. We conclude that the observed variability among these single-cell transcriptomes is derived from noncoding regions, therefore, emphasizing another layer of gene regulation essential for neuronal differentiation. In summary, the proposed approach allows high-throughput and cost-efficient mapping of complex neural circuits. Here, we will illustrate unique opportunities for unbiased molecular classification of neurons within the entire brain toward formation of the Natural System of Neurons or NeuroSystematics. We also provided the first unbiassed census of cell types in the brain, classification of neurons and synapses in complex circuits and reconstruct the evolutionary origin of neuronal phenotypes from ancestral cell types. Broad comparative and integrative genomic analyses suggest that neurons and complex brains can be evolved more than once.

Disclosures: **L.L. Moroz:** None. **G. Meredith:** A. Employment/Salary (full or part-time):; ThermoFisher. **Y. Sun:** A. Employment/Salary (full or part-time):; ThermoFisher. **D. Dhingra:** A. Employment/Salary (full or part-time):; ThermoFisher. **K.M. Candelario:** None. **L. Ianov:** None. **A. Rani:** None. **A. Kumar:** None. **D.A. Steindler:** None. **T. Foster:** None. **A. Kohn:** None. **C.J. Frazier:** None. **S. Harden:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Topic: F.02. Animal Cognition and Behavior

Support: University of Illinois Behavioral Neuroscience Fellowship

Title: The role of SHANK in trace associative learning-induced neocortical synaptic plasticity

Authors: ***S. M. COLLINS**, R. GALVEZ
Psychology, Univ. of Illinois, Urbana, IL

Abstract: 2014 SfN Abstract The role of SHANK in trace associative learning-induced neocortical synaptic plasticity It has been well established that learning induces synaptic modification in the neocortex. To examine the molecular mechanism(s) mediating this process we have utilized the associative learning paradigm whisker trace eyeblink conditioning (WTEB). During WTEB, subjects are presented with a neutral whisker stimulation (CS) which is paired

with a mild periorbital eye-shock (US). These stimuli are separated by a trace interval in which no stimulation occurs. After multiple CS-US pairings, subjects learn that the CS predicts the US and elicit an eyeblink after the CS but before US onset. Utilizing this paradigm, it has been shown that primary somatosensory cortex is required for both acquiring and retrieval of the learned association. In examining the neuronal mechanisms(s) mediating this learning event, our laboratory has demonstrated that spines in layer IV of the barrel cortex exhibit a transient increase across different phases of learning. The current study set out to determine SHANK's role in mediating this neocortical synaptic plasticity. SHANK1 and SHANK2 are of particular interest due to their localization in the post synaptic density (PSD) of excitatory neurons. These proteins indirectly bind to NMDAR, AMPAR, and mGluR; thus playing a role in glutamate receptor organization of the PSD. Furthermore, knockout of either SHANK1 or SHANK2 results in various behavioral impairments, and dysregulation of spine morphology. These findings suggest that SHANK is involved in learning induced synaptic plasticity, and thus plays a role in WTEB induced neocortical spine modification. To explore SHNAK's role in WTEB, adult C57BL/6 mice were randomly assigned to one of seven groups (acquisition, learned, over-trained, stimulus matched unpaired controls (acq-cont, learn-cont, ot-cont), or cage controls). Once subjects met the perspective behavioral criterion for their group, they were perfused and brains were collected for postmortem analyses of layer specific expression of SHANK1 and SHANK2 in primary somatosensory cortex. These analyses will begin to decipher the molecular mechanism(s) mediating neocortical learning induced synaptic plasticity.

Disclosures: S.M. Collins: None. **R. Galvez:** None.

Poster

464. Learning and Memory: Signaling and Gene Expression

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Program#/Poster#: 464.26/UU21

Topic: F.02. Animal Cognition and Behavior

Title: The role of injury-induced neurogenesis in pattern separation and completion in brain remodeling

Authors: *T.-S. YU, S. G. KERNIE
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Abstract: Pattern separation and completion are known to play important roles in associating and specifying particular events with similar environments in our daily lives. The dentate gyrus

and CA3 regions in the hippocampus are the primary brain areas where these processes occur. Adult neurogenesis in the dentate gyrus has been demonstrated participating in pattern separation and sharpening episodic memory. Traumatic brain injury patients usually suffer deficits in memory and cognition that may be due to an imbalance between pattern separation and completion that has been proposed as a potential mechanism in maintaining a healthy mental status and adult neurogenesis appears to play a critical role. We have previously demonstrated that hippocampal neurogenesis is activated in a mouse model of traumatic brain injury, and that inhibition of injury-induced neurogenesis results in deficits in cognitive recovery. To reveal the role of injury-induced newborn neurons specifically in pattern separation, we used controlled cortical injury as a brain injury model on previously generated nestin-delta-HSV-TK transgenic mice. Half of mice were fed with valganciclovir chow to inhibit injury-induced neurogenesis and half with normal chow as controls. Four weeks after surgery, mice were used to perform fear discrimination task to test the ability in pattern separation. Mice were exposed in a chamber (Chamber A) with certain contexts for 3 minutes. After 3 minutes, mice received a 2-second footshock. Mice were exposed in Chamber A once per day for consecutive 5 days. Once the mice were associated with the contexts in chamber A with a footshock, freezing behaviors were quantified. The percentage of freezing time was used as an indicator of successful association. Starting from day 6, in addition to exposure in Chamber A, mice were exposed to another chamber (Chamber B) with different contexts from chamber A and no footshock for 3 minutes. The sequences of exposures in 2 similar chambers were counterbalanced. The mice were trained to distinguish 2 similar chambers for 14 days. During the first 5 days, sham-operated or injured mice fed with normal or valganciclovir chow successfully associated Chamber A with the footshock. Although the frozen time decreased significantly in all experimental groups at day 6, when they were first exposed to chamber B, only controls (Sham, normal chow) continually learned to distinguish Chamber B from A. All three other experimental groups failed. This indicates that brain injury resulted in deficits in pattern separation and injury-induced neurogenesis is a key factor in separating the similar contexts.

Disclosures: **T. Yu:** None. **S.G. Kernie:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Support: NIMH (RO1MH102841)

Walter F. Heiligenberg Professorship

NIMH (1ZIAMH002784)

Title: Behavioral discrimination and network pattern separation can occur in the absence of neurogenesis

Authors: *V. C. PIATTI[1], Y. AN[1], M. APPALARAJU[1], S. N. GILLET[1], H. A. CAMERON[3], S. LEUTGEB[1,2], J. K. LEUTGEB[1]
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Abstract: The dentate gyrus (DG) is a unique region of the mammalian brain in that new neurons are continuously integrated into a fully functional neural circuit throughout life. Although only a small portion of the DG network at any given time is composed of immature adult-born neurons, their high excitability has motivated the hypothesis that they play an essential role in DG network function and, as a consequence, dentate-dependent behavior. We tested this hypothesis in a transgenic rat model (GFAP-TK) in which neurogenesis (NG) can be selectively blocked by the oral administration of valganciclovir (Snyder et al., SFN 2011). We confirmed with doublecortin immunohistochemistry that after 7 weeks of continual drug administration, NG was reduced by 98 ± 0.5 % in GFAP-TK$^+$ rats with drug compared to controls. Using animals with this level of reduction, we first tested the role of NG in a dentate-dependent spatial discrimination task (Morris et al., 2012). We compare rats without NG to two control groups: 1) GFAP-TK$^-$ littermate controls with drug and 2) GFAP-TK$^+$ controls without drug. In addition, we also performed colchicine-DG lesions (N = 8) to confirm that the DG is essential for distinguishing between similar spatial locations in our experimental conditions ($p = 0.02$, compared to controls, T-test). When testing GFAP-TK$^+$ rats under drug administration (N = 8), we found that the rats without NG performed at the same level as controls and were significantly better than DG-lesioned rats ($p = 0.03$, compared to DG-lesions, T-test). Our results demonstrate that immature neurons are not necessary for dentate-dependent behavioral discrimination. Next, to determine the effects of removing NG on dentate network function, we recorded single units in the DG of awake behaving GFAP-TK$^+$ rats in which we confirmed that NG was essentially absent (a reduction of 99 ± 0.5%). In rats without immature neurons, place cells did not differ substantially in firing rate, place field size, spatial information, or number of place fields compared to control rats. This finding is inconsistent with the hypothesis that immature neurons are the only active cell population in the dentate network (Alme et al., 2010). We are now testing to what extent immature neurons contribute to pattern separation and whether similar sensory inputs become less orthogonalized in a dentate network without NG. Preliminary data suggests that dentate networks without NG discriminate to the same degree as

those with intact NG. Our results suggest that immature neurons are not necessary for the DG network to perform pattern separation or to support dentate-dependent discrimination tasks.

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Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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NIH P01AG09973

Title: Hippocampal spatial coding in aged rats is altered by septal inactivation

Authors: B. L. BOUBLIL[1], M. P. BRANDON[1], M. GALLAGHER[2], J. K. LEUTGEB[1], *S. LEUTGEB[1,3]
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Abstract: The entorhinal cortex and hippocampus are required for spatial and memory processing, and these regions are connected to each other by forward connections from the superficial layers of the entorhinal cortex to the hippocampus and by backprojections from the hippocampus to the entorhinal cortex. Many models have proposed that the large proportion of grid cells in the superficial layers of entorhinal cortex provides the predominant spatial input to the hippocampus. In contrast to this hypothesis, we have shown that the spatial coding of entorhinal grid cells diminished while hippocampal spatial coding remained intact during inactivation of the medial septal area - a subcortical region that provides cholinergic, GABAergic, and glutamatergic inputs. In these experiments, place fields remained at consistent locations and retained their peak firing rates in familiar environments. When testing in novel environments during inactivation, completely distinct spatial representations emerged, and these

representations remained unchanged after grid cell firing had recovered (Brandon et al., 2014). These results suggest that entorhinal cell populations other than grid cells are sufficient for hippocampal spatial coding. In aged rats, the strength of the connections from entorhinal cortex to the hippocampus is reduced, and entorhinal cell populations undergo molecular changes, including the loss of Reelin. We asked whether such partially reduced circuit function may result in a neuronal network that can compensate to a lesser extent for the loss of subcortical projections. To test for this, we inactivated the medial septal area in aged rats in an experimental design that included two baseline 10-min recording sessions in a familiar environment, a recording session in the familiar environment immediately after the inactivation, three recording sessions in a novel environment, and additional recording sessions in the familiar environment during recovery. While most aspects of spatial coding remained intact in young rats, we found that spatial coding in aged rats was partially altered even in a familiar environment after septal inactivation. Furthermore, spatial firing patterns in novel environments did not emerge to the same extent as in control conditions. These results suggest that age-related changes in the entorhino-hippocampal circuitry have substantial impact on the mechanisms that support hippocampal spatial coding, such that cortical circuits in aged animals can no longer compensate for the loss of inputs from the medial septal area.

Disclosures: B.L. Boublil: None. **S. Leutgeb:** None. **M.P. Brandon:** None. **M. Gallagher:** None. **J.K. Leutgeb:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Walter F. Heiligenberg

Title: Impaired dentate gyrus function in early stages of a chronic model of temporal lobe epilepsy

Authors: *L. A. EWELL[1], V. LAM[1], A. MCGINNESS[1], R. KUK[1], T. GOLDRING[1], V. C. PIATTI[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1]
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Abstract: During epileptogenesis, the hippocampus undergoes severe structural reorganization, which is especially profound in the dentate gyrus (DG). In health, the DG performs pattern separation, the process of encoding similar experiences with distinct and separate neural codes during learning. We hypothesize that reorganization of the DG during epileptogenesis would undermine DG mediated pattern separation, which could be used as an early indicator that epileptogenesis is occurring. To test this we employed the low-dose kainate model for chronic epilepsy, in which dentate reorganization by way of cell loss and mossy fiber sprouting has been characterized to begin after insult and increase during epileptogenesis leading to the chronic phase marked by spontaneous seizures. Four months after induction, we tested animals on a DG dependent behavioral pattern separation task (Morris et al., 2012) and then assayed structural changes post-hoc. During the span of behavioral testing no seizures were witnessed in any of the induced animals. Induced rats (n=8) took significantly more trials to distinguish between two adjacent arms on an 8 radial-arm maze than control rats (n=5) ($p \leq 0.01$), and took the same number of trials as animals with colchicine-DG lesions. The deficit did not reflect gross hippocampal dysfunction because there was no difference between induced (n=5) and control rats (n=3) in the number of trials required for distinguishing non-adjacent arms, a paradigm that is also spared in animals with dentate lesions. Despite finding a behavioral deficit, we did not observe significant aberrant axonal sprouting or cell death at the time-point when behavioral testing occurred. These findings suggest that DG function is impaired early in epileptogenesis, before major structural change can be measured, which potentially validates the use of behavioral pattern separation tests in patients as a marker for ongoing epileptogenic processes. When examining DG network encoding mechanisms in awake behaving epileptic animals, we have found that DG neurons in chronically epileptic animals display unstable spatial coding. Spatial correlation coefficients between place fields formed during subsequent foraging sessions in the same environment were lower for epileptic animals (0.45 ± 0.1, n = 17 neurons) compared to control animals (0.76 ± 0.05, n = 24 neurons) ($p \leq 0.002$). We are currently recording from animals in the period when behavioral pattern separation was impaired to determine whether such spatial instability could account for deficits in behavioral pattern separation and to characterize changes in dentate network function during epileptogenesis.

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Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Walter F. Heiligenberg Professorship

Title: Dentate gyrus granule cells support reward-related ripple activity in the hippocampal CA3 network during spatial working memory

Authors: *T. SASAKI[1], E. HWAUN[2], V. C. PIATTI[2], S. LEUTGEB[2,3], J. K. LEUTGEB[2]
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Abstract: The hippocampal formation has been suggested to play a critical role in the formation of working memory (WM), an ability to temporally store and process relevant information for planning of future behavior. We have recently determined that dentate granule cells along the longitudinal axis of the dentate gyrus (DG) increased their firing rates specifically at reward locations in a spatial WM task and that disruption of these cells reduced behavioral performance (Piatti et al., SFN 2013). To further explore the neural mechanisms underlying DG-dependent working memory, we monitored the activity patterns of CA3 pyramidal neurons in the absence of input from the DG while rats performed a spatial working memory task on an 8-arm radial maze. Dentate granule neurons were selectively lesioned with colchicine after learning, and the loss of input was quantified using a TIMM stain to label intact mossy fiber axons. The degree of mossy fiber innervation was scored for each recording site in the hippocampal CA3 subregion. Rats with complete dentate lesions exhibited a significant impairment in spatial WM performance relative to control rats. As a possible neurophysiological correlate of WM, we next focused on sharp wave ripple complexes (SWRs) based on the recent observation that disrupting SWRs in awake animals impaired subsequent behavioral performance in a spatial working memory task (Jadhav et al., 2012). In control group, we found that the frequency of ripple events increased when the rats both received reward at the end of each goal arm and paused at the stem, the choice point of the maze. In DG-lesioned rats, there was an elimination of reward-associated ripples in recording sites determined to lack mossy fiber innervation ($t11 = 1.28$, $P = 0.23$), whereas the frequency and quality of stem-associated ripples remained unchanged ($t11 = 2.80$, $P = 0.01$). Recording sites with intact mossy fiber innervation showed a significant increase in

reward-related ripple activity (t3 = 2.82, P = 0.03). These observations suggest that direct input from the dentate gyrus is necessary to support CA3 ripple events associated with reward. Taken together, these results suggest that DG granule cells are instrumental in setting the reward-triggered network states in the hippocampus and the dynamic temporal patterns may underlie the mechanisms of DG-dependent spatial working memory.

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Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Walter F. Heiligenberg Professorship

UCSD Neurosciences Graduate Program Training Grant (2T32NS061847-06)

Title: Coding contextual changes through remapping in the medial entorhinal cortex

Authors: *G. W. DIEHL[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1]
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Abstract: The medial temporal lobe plays a critical role in the formation and storage of episodic memories. In rodents, spatial information represents a central component of these processes, as illustrated by the abundance of spatially selective place cells in the hippocampus, and grid cells in the medial entorhinal cortex (MEC). In hippocampal place cells, this robust spatial coding is further utilized to encode contextual information, with single neuronal populations capable of representing multiple contextual environments. Following large changes to an environment, the 'global remapping' of place cells is accompanied by realignment of the fields in MEC grid cells. Yet after smaller changes, such as modifications of cue configurations within an environment, the spatial firing of grid cells remains unchanged (Fyhn et al., Nature, 2007) even though hippocampal place cells are known to respond to these manipulations predominantly through the

process of ‘rate remapping’. Because of the lack of change in grid cells, it has been proposed that rate remapping in the hippocampus emerges from the intrahippocampal processing of lateral entorhinal cortex inputs. However, whether or not remapping exists upstream of the hippocampus in MEC cells that are not grid cells remains less clear. To further investigate how the MEC responds to contextual changes, we recorded the activity of single units in the superficial layers of MEC while rats explored familiar environments during context changes previously shown to induce hippocampal rate remapping (square vs. circular enclosure shape). We find that the MEC contains a population of cells, distinct from the grid cell population, that undergo remapping in response to contextual change. Unlike the field realignment seen in grid cells, these ‘context cells’ maintain stable fields with firing rates that vary based on the contextual environment, analogous to the rate remapping seen in hippocampal place cells. These cells could act, either in conjunction with grid cell changes or as an independent population, to relay from the MEC the contextual information necessary to induce remapping in hippocampal place cells. Alternatively, they may reflect the presence of a common response to context that is maintained in parallel throughout the entorhinal-hippocampal loop.

Disclosures: G.W. Diehl: None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

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Title: Optogenetic stimulation of parvalbumin-positive neurons provides an external pacemaker of hippocampal theta dynamics

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Abstract: Physiological and modeling studies suggest that theta oscillations temporally coordinate cell assemblies in the hippocampus to support memory function. Individual place cells oscillate slightly faster (9-12 Hz) than network theta oscillations (7-9 Hz), resulting in a phenomenon known as phase precession. A key question that phase precession models must address is, "How does a network of many 9-12 Hz oscillators generate a 7-9 Hz oscillation?". This has been addressed by two mechanistically distinct proposals. In one group of models, an external pacemaker provides a baseline 7-9 Hz oscillation to the entire network, and then an additional mechanism serves to increase the oscillation frequency of each neuron to cause precession through its respective place field. In another group of models, the baseline network oscillation is not provided by an external pacemaker, but instead is simply a result of a fixed temporal offset between hippocampal assemblies that oscillate 9-12 Hz. To confront these models, we used an optogenetic strategy to gain control over the medial septum, a region in the basal forebrain that has been proposed to function as the external pacemaker of hippocampal theta dynamics. 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 cells in the medial septum. We found that rhythmic stimulation of these neurons directly controlled the frequency of theta oscillations in the hippocampus, subiculum, and medial entorhinal cortex as animals explored the home cage and linear track. Place fields were unchanged during optogenetic stimulation of the septum, which allowed us to examine whether individual place cells would maintain phase precession when theta frequency was controlled experimentally. Of the neurons that showed phase precession in baseline conditions, many retained 'phase precession' at imposed theta frequencies, even during frequencies that exceed the endogenous range (> 10 Hz). These data constrain models that do not require an external pacemaker. These models would need a mechanism by which parvalbumin neurons in the medial septum proportionately govern the oscillation frequency or temporal offset of place cell assemblies, which by definition suggests that the septum functions as an external pacemaker. Our data support the prediction that the medial septum serves as a pacemaker of the theta rhythm, and suggest that our manipulation of this projection taps into the endogenous mechanism that increases the oscillation frequency of hippocampal cells in their place fields.

Disclosures: M.P. Brandon: None. **M. Donegan:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Imaging place cell dendrites during navigation

Authors: *M. SHEFFIELD, D. A. DOMBECK
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Abstract: Establishing the hippocampal cellular ensemble that represents an animal's environment involves the emergence and disappearance of place fields in specific CA1 pyramidal neurons, and the acquisition of different spatial firing properties across the active population. Basic integrate-and-fire models of place firing propose that such flexibility and diversity result solely from varying inputs to place cells, while recent studies instead suggest that place cells themselves may be playing an active role through regenerative dendritic events which can lead to synaptic plasticity and input amplification. However, due to the difficulty of performing functional recordings from place cell dendrites, no direct evidence of regenerative dendritic events exists, leaving any possible connection to place coding unknown. We used a multi-plane two-photon microscope to co-image CA1 place cell somata and dendrites expressing GCaMP6. During imaging mice navigated a virtual environment allowing us to measure place field characteristics and associated dendritic activity. We found that regenerative dendritic events do exist in place cells of behaving mice and, surprisingly, their prevalence throughout the arbor is highly spatiotemporally variable. At one end of the spectrum we found all dendritic branches could display place field transients along with the soma. However, on many occasions we found none of the branches showed calcium transients even when the soma did. The extent to which place cell dendrites expressed calcium transients in the place field was associated with the precision of the place field and its stability over days. This suggests that the dynamics of spiking throughout the dendritic arbor may play a key role in forming the hippocampal representation of space.

Disclosures: M. Sheffield: None. **D.A. Dombeck:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Cellular resolution optical imaging of medial entorhinal cortex

Authors: *J. G. HEYS, D. A. DOMBECK
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Abstract: Lesion experiments in rodents have demonstrated that the medial entorhinal cortex (MEC) is important for spatial navigation and spatial memory (Steffenach et al. 2005). Consistent with these observations, electrophysiological recording experiments have shown that several distinct functional classes of spatially selective neurons exist in MEC (Fyhn et al. 2004; Hafting et al., 2005; Sargolini et al., 2006; Solstad et al. 2008). In particular, a functional class of neurons in MEC, termed grid cells, fire selectively when an animal visits the vertices of a regular repeating triangular lattice, tiling the floor of an environment (Fyhn et al., 2004; Hafting et al., 2005). To date, all cellular resolution experiments characterizing grid cells have used extracellular or intracellular recording techniques. In other brain regions, cellular resolution optical imaging methods have been developed and applied to provide additional and complementary descriptions of the neural circuitry. Previous methods developed to image hippocampal place cells in virtual reality require positioning the microscope above a window that has been surgically implanted directly above the hippocampus (Dombeck et al. 2010). However, due to the deep position of the entorhinal cortex in the brain and the overlying transverse sinus, this direct imaging approach cannot be used and thus so far it has not been possible to use cellular resolution imaging methods to study the physiology of MEC *in vivo*. To overcome these problems, we have developed a novel optical and surgical approach to allow for the first cellular and sub-cellular resolution imaging of grid cells. Our preliminary data show grid field firing properties that are consistent with previously published intra and extra cellular electrophysiological recordings of grid cells.

Disclosures: J.G. Heys: None. **D.A. Dombeck:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

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Title: Hippocampal ensemble representations of the learning context are critical for resolving interference

Authors: *D. A. BULKIN, L. M. LAW, D. M. SMITH
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Abstract: The ability to resolve interference is essential for learning to be useful. A ubiquitous solution, identified throughout the animal kingdom, is to group memories based on the context where they were learned. Returning to a familiar context automatically triggers memories that were learned there, while preventing intrusions from context inappropriate memories that were learned elsewhere. Widespread evidence indicates that the hippocampus plays a critical role in representing the context, and recent work from our group has demonstrated that an intact hippocampus is essential for using the context to overcome interference. Precisely how hippocampal activity supports interference-free recall has remained unclear. One possible mechanism is that hippocampal ensembles prime the retrieval of context appropriate memories, thereby inhibiting the retrieval of context inappropriate memories. This framework suggests that hippocampal ensembles change their firing pattern with context (a phenomenon that has been extensively demonstrated), and that this change is associated with improved performance in the face of interference. In the present study, we recorded the activity of ensembles of neurons in the dorsal hippocampus while rats learned a proactive interference task. Rats learned two sets of conflicting odor discrimination problems either in the same context, or in two distinct contexts. Rats that learned both problem sets in the same context showed impaired performance while learning the new problems. These rats showed hippocampal representations that did not change, presumably their hippocampal representations primed incorrect memories from the old problem set, resulting in significant interference. In contrast, rats that learned the second problem set in a new context exhibited entirely new hippocampal representations and performance was enhanced relative to those learning in the same context. Interestingly, there was considerable trial to trial variation in hippocampal activity. We assessed the similarity of each set 2 trial's ensemble representation to the representations on set 1 trials. Remarkably, in both the same and different context conditions, errors rarely occurred on trials where the hippocampus showed a distinct representation. Instead, errors occurred almost exclusively when the old representation was more active (i.e. greater similarity to set 1 in ensemble activity), suggesting that the presence of a distinct new representation mitigated the effects of interference.

Disclosures: D.A. Bulkin: None. **L.M. Law:** None. **D.M. Smith:** None.

Poster

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Title: Neurons in the retrosplenial cortex encode navigational cues and reward locations

Authors: *L. C. VEDDER, D. M. SMITH
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Abstract: The retrosplenial cortex (RSC) is reciprocally interconnected with the hippocampus and anterior thalamus and, like these regions, is critically important for a variety of learning and memory processes, including spatial navigation, contextual memory and episodic memory. Previous studies have shown that the RSC is necessary for processing visual and auditory cues in behavioral assays of learning and memory in animals. The RSC also processes navigational cues, such as landmarks, which inform subjects of goal locations. Previously, we found that more than 40% of the neurons in the rat RSC respond selectively to the conjunction of a reward and its location during the first training session when that conjunction also served as a critical cue that informed the rat of the location for the next reward (i.e. when rats used a win-stay strategy, Smith et al, 2012, Hippocampus, 22(5):1121-33). However, because the reward location was itself a navigational cue, we could not distinguish between responses to reinforcing stimuli and responses to navigational cues that signaled the location of upcoming rewards. In the present study, we separated these components by signaling the reward location with a light cue at the beginning of the trial, several seconds before the rat arrived at the reward. We used a conditional T-maze task in which flashing LED lights over the reward cups cued the location of a chocolate milk reward. In each session, rats were given 40 trials, 20 left and 20 right in an unpredictable order. We recorded single unit activity in the RSC during learning and asymptotic performance in this task. The rats learned this task in 6.33 ± 0.49 sessions (mean ± SEM). 60% of the neurons in the RSC developed responses to the light cue during the first day of training, before the rats exhibited behavioral evidence of learning. When rats reached asymptotic levels of performance, a subset of the RSC neurons (15%) responded selectively to the conjunction of the light cue and its location. We also found that RSC neurons developed responses to the conjunction of reward and its location over the course of training, with 57% of RSC neurons exhibiting these responses at asymptote. Our results indicate that RSC neurons rapidly encode critical navigational cues, whereas reward responses develop more slowly over the course of learning. These findings are consistent with the hypothesis that the RSC contributes information about potential navigational cues to a broader memory and navigation network.

Disclosures: **L.C. Vedder:** None. **D.M. Smith:** None.

Poster

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Title: Representations of cues and space in the retrosplenial cortex during continuous spatial alternation

Authors: ***A. M. MILLER**, W. MAU, S. PARAUDA, K. YU, D. M. SMITH
Cornell Univ., Ithaca, NY

Abstract: The retrosplenial cortex (RSC) plays a prominent role in spatial learning and memory. The RSC is interconnected with the hippocampal formation and shares a number of functions with the hippocampus including spatial and contextual memory. In line with neuropsychological data showing that damage to either the RSC or the hippocampus in humans causes spatial impairments and amnesia, work with rats has shown that lesions to either structure result in impairments on spatial tasks such as the Morris water maze and the radial arm maze, as well as a number of conditioning tasks such as discriminative avoidance learning and contextual fear conditioning. One explanation for the wide range of behavioral impairments is that the RSC may play a role in processing behaviorally relevant cues and the spatial relationships among them. In support of this idea, we have previously shown that up to 40% of RSC neurons encode reward locations when they serve as critical navigation cues in a blocked spatial alternation task (Smith et al, 2012, Hippocampus, doi: 10.1002). However, the firing properties of RSC neurons have not been described in a task known to depend on the RSC. Here, we recorded in the RSC of rats during training on a continuous spatial alternation task that we have previously shown to require the RSC (Miller & Smith, 2012). In this task, rats must remember which reward location they visited on the previous trial in order to visit the opposite reward location on the current trial (i.e., alternation). Prior to training, a microdrive with 16 independently movable tetrode recording electrodes was implanted bilaterally over the granular b region of the RSC. Recording began on the first day of training and continued through asymptotic performance. Consistent with previous results, we found that over a third (36.04%) of RSC neurons uniquely encoded each reward location, and that the proportion of these cells was similar during the first, middle, and last

training sessions ($\chi 2(2) = 1.06$, $p = 0.59$). Additionally, we found that many RSC neurons fired differently on the two sides of the maze, and that the proportion of these cells increased over the course of training ($\chi 2(2) = 13.2$, $p < 0.01$). Preliminary population analyses further indicated that neural representations of the left and right reward areas became progressively more distinct as rats learned the task (i.e., Mahalanobis distance increased with training). These findings provide additional support for the role of the RSC in spatial representation and the processing of behaviorally significant cues.

Disclosures: **A.M. Miller:** None. **W. Mau:** None. **S. Parauda:** None. **K. Yu:** None. **D.M. Smith:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 465.12/UU33

Topic: F.02. Animal Cognition and Behavior

Support: hhmi

Title: Synchronous increase of neuronal excitability is required for the generation of specific hippocampal neuronal sequences

Authors: Z. ROTH[1], Y. WANG[2], S. ROMANI[3], *E. PASTALKOVA[2]
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Abstract: Hippocampal place cells are activated one after another at the time scale of seconds whenever an animal traverses place fields of these neurons in a given environment. The same place cells are activated in the same order during short sequences in the course of individual cycles of theta oscillation and sharp-wave/ripple events. These compressed sequences are thought to be important for the consolidation of the slow, place field sequences but how the compressed sequences are generated is not known. Both, individual theta cycles and sharp-wave events are characterized by synchronized increase in the excitability of a large population of neurons. Is this synchronized increase of excitability the necessary condition for the generation of the compressed neuronal sequences? We characterized the frequency with which hippocampal neurons were sequentially activated in the same order as during the traversal of the maze in the course of theta and ripple events before and after we manipulated the amount of synchrony

during theta and sharp-wave events by inactivating medial septum. Medial septum inactivation almost entirely eliminated the synchronized change of excitability during theta state and at the same time it increased the level of the population excitability during sharp-waves. Correspondingly, we found that significantly less place field sequences were activated during theta state while significantly more place fields sequences were activated during ripple state. We propose a network model, which can reproduce these experimental results and suggests that synchronized increase of the population excitability at the beginning of the individual cycles of theta and ripples are necessary for the initiation of the sequential firing of hippocampal neurons.

Disclosures: **Z. Roth:** None. **Y. Wang:** None. **S. Romani:** None. **E. Pastalkova:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 465.13/UU34

Topic: F.02. Animal Cognition and Behavior

Support: HHMI and affiliation Janelia Farm Research Campus 19700 Helix Drive 20147 Ashburn VA

Title: Sensory cues are insufficient to evoke hippocampal place fields in a novel environment

Authors: ***Y. WANG**, A. LEONARDO, E. PASTALKOVA
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Abstract: The hippocampus is a brain structure necessary for spatial learning and memory. Hippocampal neurons fire at specific locations in an environment. Although the firing rate of these 'place cells' is modulated by theta rhythm, elimination of theta has little effect on the spatial tuning of place cells in a familiar environment. In contrast, the same manipulation impairs spatial learning in a novel environment, suggesting theta rhythm might play a critical role in place field formation. To explore the dependence of place cell formation on theta rhythm, we inactivated medial septum and found that virtually no place fields were formed in a novel environment. In addition, place fields that formed under normal conditions in a novel environment disintegrated once medial septum was inactivated. Thus, intrinsic network activity paced by the medial septum, rather than sensory cues, governs the formation of place fields.

Disclosures: **Y. Wang:** None. **A. Leonardo:** None. **E. Pastalkova:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 465.14/UU35

Topic: F.02. Animal Cognition and Behavior

Support: Stiftung Mercator

International Graduate School of Neuroscience

Title: Imaging the aging memory trace with high resolution in the medial temporal lobe

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Abstract: While the standard model of consolidation proposes a transfer of the memory trace from the hippocampus to the cortex over time, the multiple trace theory suggests a permanent role of the hippocampus in the retrieval of recent and remote memories. Despite the growing evidence for a functional segregation of the hippocampal subfields CA1 and CA3, it remains unclear how these areas contribute to memory retrieval. Furthermore, recent studies have suggested a critical role of the parahippocampal region in the retrieval of remote memories, but the specific contribution of the perirhinal, postrhinal and lateral and medial entorhinal cortices is not known. Here, we used contextual-fear conditioning and imaged the memory trace over a year (half the life span of a mouse) in the different MTL areas. For this purpose, we used a high-resolution molecular imaging technique based on the detection of the immediate-early gene Arc, which served as a marker of cell activation and allowed for the identification of each cell activated during memory retrieval (here: after 1 day, 1 week, 1 month, 6 months and a year) Apart from revealing a differential recruitment of the hippocampal and parahippocampal subareas, the data show not only a functional segregation between CA1 and CA3, but also selective contributions of the different parahippocampal areas as the memory trace ages. These data appear to, at least partially, reconcile the two opposing theories of memory consolidation.

Disclosures: V. Lux: None. **M. Sauvage:** None. **T. Kitsukawa:** None.

Poster

465. Cortical and Hippocampal Circuits: Learning and Memory

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 465.15/UU36

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIMH grant MH094360-01A1

Title: Neural networks of the mouse entorhinal cortex

Authors: *M. S. BIENKOWSKI[1], M. Y. SONG[1], I. BOWMAN[1], M. BAY[1], L. GOU[1], B. ZINGG[2], H. HINTIRYAN[1], N. N. FOSTER[1], A. W. TOGA[1], H.-W. DONG[1]
[1]Inst. for Neuroimaging and Informatics, [2]Zilkha Neurogenetic Inst., USC, Los Angeles, CA

Abstract: At the caudal end of the temporal lobe, the entorhinal cortex (ENT) provides a critical gateway for communication between the forebrain and the hippocampus. The perforant path, arising from ENT neurons in layers 2 and 3, provides the major cortical input to the hippocampus. In turn, the deep layers of the ENT are a major target of hippocampal output and ENT neurons within the deep layers project broadly throughout the cortex. Indeed, in our recent computational analysis of the neural networks of the mouse neocortex, the connectivity of the ENT stood out as a critical hub connecting cortical subnetworks across hemispheres (Zingg et al., 2014). However, the ENT constitutes one of the largest cortical areas of the rodent brain and this broad connectivity is not indicative of the entire ENT region. The ENT can be divided into a medial (ENTm) and lateral (ENTl) region and the ENTm and its grid cells have a well-defined role in spatial navigation (Moser et al., 2008). While some studies have attempted to further subdivide and investigate ENT subregion connectivity in the rat (Insausti et al., 1997, Agster and Burwell, 2009), this regional connectivity has not been fully examined in the mouse and ENTm and ENTl subregions are not defined by the commonly used mouse Allen Reference Atlas (ARA). Using retrograde and anterograde tracer data generated from over 150 double coinjections as part of the Mouse Connectome Project, the goal of this study was to comprehensively dissect mouse ENT subdivisions based on connectivity and cytoarchitecture, characterize each subdivision's laminar connectivity, and perform network analyses of the ENT's connectivity to better understand its role in brain-wide neural networks. First, using Nissl-stained sections from the mouse ARA, we outlined ENT subdivisions for each ARA atlas level following previous cytoarchitectural descriptions (Groen et al., 2001). This new ENT atlas was used to plot ENT injection sites with subregional and laminar specificity as well as analyze retrograde and anterograde labeling patterns within the ENT produced by cortical, amygdalar, thalamic, and striatal coinjections. Our results provide a comprehensive description of the unique

connectivity patterns for each layer of the ENT and its subdivisions. Ongoing network analyses of these connections and connectivity matrix construction will provide further insight into the overall role of each ENT subdivision in the mouse macroconnectome.

Disclosures: **M.S. Bienkowski:** None. **M.Y. Song:** None. **I. Bowman:** None. **M. Bay:** None. **L. Gou:** None. **B. Zingg:** None. **H. Hintiryan:** None. **N.N. Foster:** None. **A.W. Toga:** None. **H. Dong:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 466.01/UU37

Topic: F.02. Animal Cognition and Behavior

Support: CIN Intramural Project Grant

Title: ratCAVE: A novel virtual reality system for freely moving rodents

Authors: ***N. A. DEL GROSSO**[1], A. SIROTA[2]
[1]Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]BCCN and Synergy Cluster, Ludwig-Maximilians Univ., München, Germany

Abstract: Behavioral studies using freely-moving rodents are essential for associating neuronal and sensorimotor dynamics, but these paradigms traditionally come at a cost; unlike head-fixed setups, they relinquish rigorous experimental control of sensory information flow to the rodent. Complexity, reproducibility and real-time experimental control of visual cues used in studies of freely-moving rodents are very limited, while their flexible manipulation is highly important for elucidation of the mechanisms of sensorimotor processing and spatial coding (Jezek et al 2011; Furtak et al 2009). Virtual reality systems (VRS) overcome these limitations, since they allow experimenters to flexibly manipulate the rodent's sensorimotor feedback using computer-generated visual stimuli (Hölscher et al, 2005). However, current rodent VRS rely on head- or body-fixed treadmill navigation, which requires training, limits natural behavior and sensory afferents giving rise to neuronal dynamics not fully comparable to that observed in freely moving animals (Harvey et al 2009; Ravassard et al, 2013). We demonstrate here a novel computer-assisted virtual environment (CAVE) setup that allows freely-moving rodents to navigate and interact with virtual environments, which we coin ratCAVE. The rodent’s head position is tracked in real-time using a reflective marker-based, multi-camera system. The virtual

environment is front-projected onto the walls and floor of the arena using projection-mapping techniques. This image is then dynamically warped to appear geometrically correct from the rat's perspective. Our freely-moving VR approach adds motion parallax, head-direction, and vestibular cues to those three-dimensional sensory cues currently available in head-fixed VR setups. We demonstrate the benefits of this approach using conventional tasks devised for freely moving rats. Our freely-moving VR approach combines ethological behaviour with close to natural idiothetic and 3D visual feedback, while providing unprecedented level of experimental control of visual environment. ratCAVE opens new level of analysis of spatial navigation and learning mechanisms.

Disclosures: **N.A. Del Grosso:** None. **A. Sirota:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 466.02/UU38

Topic: F.02. Animal Cognition and Behavior

Support: FCT fellowship SFRH/BPD/82220/2011

Title: Joint analysis of cholinergic tone and network dynamics in cortical and hippocampal neuronal networks *in vivo* using enzyme-based biosensors

Authors: ***R. M. SANTOS**[1,2,3], J. LARANJINHA[1,4], R. M. BARBOSA[1,4], A. SIROTA[2,3]
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Abstract: Acetylcholine (ACh) modulates neuronal responses, leading to wide functional effects at the network level, which include cortical desyncronisation and modulation of theta oscillations in the hippocampus. The unspecific distribution of ACh terminals and receptors suggests that its effects rely on its spatio-temporal profile and concentration. Accordingly, the dynamics of cholinergic tone relative to behavioral events and/or ongoing activity in the hippocampus is crucial to determine its functional role. Thus, sensitive measurements from multiple brain regions in behaving animal with high spatio-temporal resolution are critical to understand the mechanisms of ACh action. Spatial resolution is particularly important for measurements in

hippocampus to avoid averaging from multiple layers. In this work, we developed an enzyme-based electrochemical biosensor to assess cholinergic activity with high spatio-temporal resolution and sensitivity, by measuring extracellular choline (Ch) as a reporter of ACh release. This approach is based on the amperometric measurement of H_2O_2 generated by choline oxidase (ChOx) in the presence of Ch and O_2. The microelectrodes consisted in two side-by-side disk shaped 50 µm diameter Pt/Ir wires. This design is inexpensive, scalable and compatible with conventional tetrode drives. The small disks provide adequate spatial resolution for hippocampal measurements and the orientation of the wires optimizes rejection of noise and interferent compounds *in vivo* by sentinel channel subtraction. The Ch measuring site was coated with ChOx and chitosan cross-linked with benzoquinone. For the sentinel, ChOx was replaced by BSA. Electrochemical measurements were done using a multichannel miniature head-stage (NPI). The biosensors showed a low detection limit of 10 nM Ch and a response time of ~2 s. In anesthetized rats, local field potential features were extracted from the recording together with Ch dynamics. Sustained Ch increases during spontaneous and tail-pinch evoked theta periods reached amplitudes up to 5 µM in the cortex and 1 µM in the hippocampus CA1. Brief theta or desynchronization periods were accompanied by Ch dynamics frequently involving decreases followed by slower increases. Control experiments indicate that Ch responses were independent of physiological O_2 changes, in agreement with the low Km for O_2 of 2 µM determined *in vitro*. The data supports the reliability of the biosensors to measure cholinergic activity in the brain. Spontaneous Ch changes suggest a bidirectional modulation of cholinergic tone. We are currently combining these sensors with multichannel electrophysiology in freely moving rodents.

Disclosures: R.M. Santos: None. **J. Laranjinha:** None. **R.M. Barbosa:** None. **A. Sirota:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 466.03/UU39

Topic: F.02. Animal Cognition and Behavior

Title: Anatomical dissection of distinct high-frequency oscillations in the hippocampus

Authors: *E. RESNIK[1,2], G. SCHWESIG[1,2], N. IDE[1,2], J. GRABOSKI[1,2], A. SIROTA[1,2]
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Abstract: Hippocampal function in learning and spatial navigation relies on complex dynamic interplay between multiple afferents and local networks. Activation of each of these networks is associated with transient high frequency oscillation (FOs) events covering gamma (20-100 Hz) and epsilon (100-200 Hz) frequency ranges. FO generators generally exhibit distinct frequency content and give rise to LFP signals with specific anatomical localization dictated by axonal projections of its constituent neuronal populations and morphological and physiological properties of the target neuronal populations. Specifically, well segregated axonal termination regions and dendritic domains in hippocampal networks favor minimal anatomical overlap of oscillatory synaptic inputs produced by different FO generators. This implies that objective characterization of FO generators could rely on detection of anatomically and spectrally limited transient oscillatory events in the hippocampal LFP. Conventionally used statistics, such as average spectrum (Csicsvari et al., 2003) or phase-amplitude modulation measures (Tort et al., 2008) for a single channel LFP do not capture the complete picture and introduce various biases into characterization of FO generators and detection of respective FO events. Using multichannel LFP recordings across all the hippocampal lamina and multiple single unit recordings we detected FOs as point events, localized in time, frequency and anatomical space (Sirota et al., 2008). Analysis of frequency distributions of FOs and their laminar localization identified large number of distinct FO generators characterized by their laminar and frequency range, as well as preferred theta oscillation phase. Many of the FO generators were not seen in the mean power spectra or in phase-amplitude coupling maps. In fact this objective analysis identified moderate to strong biases in conventionally performed detection methods. Based on bivariate spectral analysis of LFP and single units in the hippocampus and the afferent medial entorhinal cortex we ascribe anatomical circuits responsible for generating these diverse FOs. Only small fraction of the FO generators is of local origin, while majority are residing in afferent hippocampal or entorhinal circuits. Moreover, some FOs similar in their properties, but detected in different anatomical layers were not independent and could be, therefore, generated by the same mechanism. Our detection and dissection approach to fast oscillatory dynamics in the hippocampus lays the foundation for future analysis of its relationship to neural population dynamics, temporal coding, as well as animal behaviour.

Disclosures: **E. Resnik:** None. **G. Schwesig:** None. **N. Ide:** None. **J. Graboski:** None. **A. Sirota:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: CIN-Weizmann Cooperation Grant

Title: Laminar analysis of neocortical gamma oscillators

Authors: *A. N. IDE[1,2], A. SIROTA[1,2]
[1]Ctr. for Integrative Neurosci. (CIN), Tübingen, Germany; [2]BCCN and Synergy Cluster, Ludwig-Maximilians Univ. München (LMU), München, Germany

Abstract: Information coding in the brain has been associated with precise timing of neuronal spike firing and internal oscillatory dynamics. This timing strongly depends on the presence of fast membrane potential fluctuations, which mostly rises from endogenous high frequency oscillations in the gamma range (30-150 Hz). Previous studies have shown that activation of local cortical networks is associated with the transient emergence of local gamma oscillations (Sirota et al 2008). To extend this work to all cortical layers we used multiple silicon probes to record extracellular field potentials and multiple single unit activity across all cortical layers in somatosensory cortex of rats during awake and sleep. Laminar analysis of neocortical gamma bursts, independently detected in time, frequency and space, revealed the existence of multiple putative gamma oscillators. These gamma bursts were spatially confined to superficial and deep layers, exhibiting distinct ranges of oscillation frequency and synchronized with local ensembles of neurons, suggesting the existence of several distinct circuits that generate these oscillations. In order to explore the mechanistic role of gamma oscillations across sleep and awake states, as well as the mechanisms responsible for the slow time scale dynamics of gamma oscillations, we characterized the modulating effect of cortical slow oscillations, hippocampal theta rhythm and optogenetic subthreshold AC modulation on gamma oscillatory dynamics. All identified neocortical gamma oscillators were temporally coordinated by the slow rhythms, but with notable difference depending on the laminar and frequency localization of the oscillator. We hypothesize that analysis of circuit-specific neocortical dynamics with respect to hippocampal theta and slow oscillations is instrumental for understanding the mechanisms of information flow via these circuits during learning and memory consolidation.

Disclosures: A.N. Ide: None. **A. Sirota:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

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Program#/Poster#: 466.05/UU41

Topic: F.02. Animal Cognition and Behavior

Support: DFG SPP 1665

CIN Intramural Project Grant

Title: Behavioral state dependence of hippocampal place cell expression in exploring rat

Authors: J. J. GRABOSKI[1], *A. M. SIROTA[2], E. RESNIK[1]
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Abstract: Rats explore their environments with numerous behavioral strategies and sensory sampling patterns, to gather information, spatially distributed throughout an environment. An allocentric cognitive map within the hippocampal network is developed during epochs of exploration, as reflected in the spatial-contextual nature of place cell activity. Traditionally, place-cell firing fields are derived from locomotive epochs (> 2-4 cm/s), without differentiation of the diverse sensory sampling patterns (i.e. complex head motions associated with olfaction, olfactory and tactile rhythmic sampling, head turns). Most experimental paradigms lead to non-uniform distributions of exploratory sub-behaviors through a constrained selection of behavioral strategies associated with a reward (Markus et al 1995; Fenton et al 2010), while variability in place cell firing is attributed to purely internal neural processes. Interestingly, analysis of rat head scans proved useful in understanding the formation of place fields (Monaco et al, 2014). To capture the complexity of exploratory behavior we tracked rats with marker-based Vicon 3D motion tracking, to capture trajectories with high spatial (~100um) and temporal (~120Hz) resolution. A QDA model was constructed and validated with hand-labeled behavioral states (e.g. walking, rearing, head turning, head-bobbing and autonomic behavior) for a few sessions, and was used to automatically segment and classify behavior across all animals and sessions. Random foraging is presumably dominated by olfactory and tactile perception, while taxon navigation is predominately a visual activity. We have found that kinematic features of the head and body can be used to split the walking behavior into two sub-types of walking differentiated by the height and pitch of the animal's head, which we speculate resemble random foraging and taxon navigation. We recorded place cells in the CA1 and CA3 of the hippocampus, which demonstrate selectivity for a specific behavioral-state, or remapping between states.

Disclosures: **J.J. Graboski:** A. Employment/Salary (full or part-time):; Center for Integrative Neuroscience University of Tuebingen, Germany, BCCN and Synergy Cluster, Ludwig-Maximilians Universität München, Germany. **A.M. Sirota:** None. **E. Resnik:** A. Employment/Salary (full or part-time):; Center for Integrative Neuroscience, University of Tuebingen, Germany, BCCN and Synergy Cluster, Ludwig-Maximilians Universität München, Germany.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 466.06/UU42

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH60013

NIMH R01 MH61492

ONR MURI N00014-10-1-0936

Title: Effects of a benzodiazepine and a serotonin 1A receptor agonist on neural activity in the rat medial entorhinal cortex

Authors: *C. K. MONAGHAN[1,2], G. CHAPMAN, IV[1], M. E. HASSELMO[1]
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Abstract: Theta rhythm is a prominent frequency range (6-10 Hz) found in oscillations of the local field potential and interspike intervals of single units throughout many areas of the rodent brain. Theta rhythm has been proposed to play a role in learning and memory and in the generation of the spatially periodic firing pattern of entorhinal grid cells. This rhythm is especially prominent throughout the medial septum, hippocampus, and medial Entorhinal Cortex (mEC), and reduction of this rhythm correlates with disruption of the periodicity of grid cells (Brandon et al., 2011; Koenig et al., 2011). Systemic administration of anxiolytics such as benzodiazepines and serotonin 1A receptor agonists has been shown to reduce the frequency of theta rhythm recorded from the hippocampus (McNaughton et al., 1986; Coop & McNaughton, 1991; Wells et al., 2013) but it is unclear if similar reductions occur in the mEC and what effects they have on single-unit activity. Here we report the effects of the benzodiazepine diazepam and the serotonin 1A receptor agonist 8-OH-DPAT on local field potential (LFP) activity and firing properties of single units, both recorded in the mEC. Both drugs reduced the frequency of theta as recorded both in the LFP and in the single unit rhythmicity seen in the spike time autocorrelation of theta rhythmic cells, while leaving other single unit firing properties largely intact. These findings complement previously reported results from hippocampal recordings and expand detection of anxiolytic drug action to the mEC.

Disclosures: C.K. Monaghan: None. **G. Chapman:** None. **M.E. Hasselmo:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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NIMH Grant R01 MH61492

NIMH Grant 1F31MH102022-01A1

ONR MURI award N00014-10-1-0936

Title: Addressing theta rhythmicity in extracellularly recorded neurons in rat and bat

Authors: *J. R. CLIMER[1,2], R. DITULLO[1,3], J. R. HINMAN[1], G. CHAPMAN, IV[1], M. P. BRANDON[5], M. E. HASSELMO[1], U. T. EDEN[4]
[1]Dept. of Psychological and Brain Sci., [2]Grad. Program for Neurosci., [3]Undergraduate Program in Neurosci., [4]Dept. of Mathematics and Statistics, Boston Univ., Boston, MA; [5]Ctr. for Neural Circuits and Behavior, Div. of Biol. Sci., UC San Diego, La Jolla, CA

Abstract: The firing of neurons in the rodent hippocampus and entorhinal cortex is strongly modulated at theta frequency. For analysis of extracellular recording data, researchers have usually relied on the spectral properties of the spike-time autocorrelogram to examine theta modulation, most notably in the "theta index" measure. Recent studies have applied these techniques to validate the presence of theta-modulation in single unit recordings in different species. It has been suggested that the theta index may be substantially biased by the firing rate of a neuron, and that an in depth examination of the theta index is warranted. Here, we have done such an examination using a large battery of real and simulated data. We found that the theta index is substantially biased by a number of features, including the peak firing rate of the neuron, the amount of time spent in the neuron's receptive field, and other properties such as theta-cycle skipping. We have developed a more statistically rigorous method of examining theta rhythmicity using maximum likelihood estimation on a parametric model of the distribution of lags, which helps to alleviate many of the challenges facing the theta index, and have applied this technique to rodent and bat grid cell recordings. Maximum likelihood estimation provides the unique advantage in that it treats each observed lag as an individual observation, and allows us to perform statistical hypothesis testing on individual parameters such as the level of rhythmicity or the peak frequency in single cells across manipulations. Because each spike is not collapsed into

the autocorrelogram, we can also better quantify the relationship between properties of the rhythmicity (i.e. peak frequency) and other variables (such as behavioral running speed). Together, these techniques promise to enrich our ability to examine rhythmic properties of extracellularly recorded single units.

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Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant MH61492

NIMH Grant 1F31MH102022-01A1

Title: Hypothesis testing of grid cell parameters using a maximum likelihood framework

Authors: ***R. DITULLIO**[1], J. R. CLIMER[2], M. E. HASSELMO[2], U. T. EDEN[3]
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Abstract: Grid cells have been a recent topic of interest in the field of neuroscience due to their implication in spatial memory and potential role in episodic memory. Grid cells are named for the distinctive, grid-like firing pattern they produce when recorded from as an animal explores an environment with the firing fields of grid cells falling on the vertices of equilateral triangles regularly tessellating throughout the environment. This unique firing pattern can be further defined by the geometric relationships between these firing fields and many studies have focused on how these geometric parameters of the fields change under various manipulations. We have previously presented on how a maximum likelihood framework can be utilized to estimate these parameters and shown an algorithm that implemented this framework with some success. We now demonstrate an updated version of that algorithm which addresses problems of the previous version and go into further detail of how this framework can be used in a hypothesis testing setting. We analyze both simulated and real data to show how parameters of interest of the grid firing field can be estimated and how confidence bounds can be placed about these estimations. These techniques offer a striking advantage in hypothesis testing: because they treat each spike

as an observation, a statistical test can be performed on an individual cell between two sessions as opposed to tens or hundreds of cells in many manipulations. We apply the hypothesis testing framework to simulated data to demonstrate the power of the test in detecting differences between parameters individually and firing fields holistically. As a proof of concept, we apply the hypothesis testing framework to previous findings to replicate the reported results. In conclusion we summarize the advantages this framework affords experimentalists and provide a resource for the code of the MATLAB implementation of the algorithm.

Disclosures: **R. Ditullio:** None. **J.R. Climer:** None. **M.E. Hasselmo:** None. **U.T. Eden:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

Title: State-dependent physiological properties in the hippocampus-amygdala networks

Authors: ***G. GIRARDEAU**, I. INEMA, A. FERNANDEZ-RUIZ, G. BUZSÁKI
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Abstract: Activity in amygdala networks during the acquisition and retrieval of classical and contextual fear conditioning has been extensively studied in rodents. However, little is known about the basic physiological properties of the amygdala and its interactions with the hippocampus during wakefulness, slow-wave-sleep and REM-sleep. Here we designed a new task combining extensive spatial sampling of a linear track with a location-specific fearful element (air puff) to investigate neuronal dynamics in the hippocampo-amygdala networks. Large neuronal ensemble recordings were performed using 4-shank and 8-shank silicon probes in the amygdala and in the dorsal hippocampus during experimental sessions including extensive sleep periods before and after training on the task. We characterized the oscillatory and firing rate dynamics in the basolateral amygdala (BLA) across vigilance states in comparison to the hippocampus. We found that wakefulness in BLA is characterized by a strong oscillatory activity in the 45-65Hz gamma band modulated by very slow (2-4Hz) oscillations. Unlike the

hippocampus, theta power in BLA during wakefulness is weak or non-existent, and firing rates in the principal (excitatory) cells are extremely low. While in the hippocampus, firing rates and oscillatory activity are similar during REM-sleep and wakefulness, gamma activity in the amygdala is strongly decreased during REM, together with a slight power increase in the theta band and a robust increase of firing rates in principal cells. Slow-wave-sleep in the BLA shows a global broad-band increase at low frequencies (0-12Hz), with firing rates in principal cells at intermediate levels. Firing rates of putative inhibitory cells remain consistently high across states. Further analysis will concentrate on modulation of amygdala cells by hippocampal ripples and theta oscillations during different behaviors.

Disclosures: G. Girardeau: None. **I. Inema:** None. **A. Fernandez-Ruiz:** None. **G. Buzsáki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

Title: Methodological insights into hippocampal replay and pre-play

Authors: *A. D. GROSMARK[1], G. GYÖRGY BUZSÁKI[2]
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Abstract: Hippocampal replay has been variously detected as the preservation from a ‘Maze’ epoch to a subsequent ‘Post’ epoch of either the pair-wise or higher-order structure in either the firing rate co-modulation or spike-timing relationships between pyramidal cells. While these methods are distinct, they are generally regarded as measuring a unitary ‘replay’ phenomena. In the current study we recorded from the CA1 hippocampal layer of rats before, during, and after the exploration of a completely novel environment. This data set was analyzed using several replay methodologies, the results of which were found to subtly diverge under a subset of conditions. While both replay, as well the more recently described 'pre-play' phenomenon, were observed, both were found to be dominated by non-local (different silicon-probe shank)

interactions. However, while replay was observed in both pair-wise and higher-order interactions, pre-play was specifically restricted to higher-order sequential interactions. The insights derived from this cross-methodological analysis may thus be significant in informing future models of both replay and pre-play.

Disclosures: A.D. Grosmark: None. **G. György Buzsáki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

Title: Noninvasive single-unit recording from the surface of the brain

Authors: *D. KHODAGHOLY[1], J. GELINAS[1], W. DOYLE[2], G. MALLIARAS[3], G. BUZSAKI[1]
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Abstract: Electronic devices that interface with living tissue have become medically important to improve diagnosis and treatments. On a more fundamental level, most breakthroughs in our understanding of the basic mechanisms of information processing in the brain have been obtained by means of recordings from implantable electrodes. There is a tremendous need for developing advanced materials solutions for the biotic/abiotic interface. Here, we demonstrate the use of a high-density conducting polymer based electrode arrays for acquiring neuronal activity at single neuron resolution from the surface of the brain in freely moving rats and human patients. The surface probe consists of 4 μm thick Parylene C that contains 256 electrodes covered by the conducting polymer poly (3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) using a generic lithographic process. The electrodes are arranged on a hexagonal lattice, with individual electrodes having an area of 12 μm x 12 μm and a center-to-center distance of 20 μm. This particular design provides a fine surface map of the electrical

activity in a brain region of interest, while also generating unprecedented single cell resolution without penetrating the brain. Multi/single unit activities were successfully recorded from the surface of rat somatosensory cortex and hippocampus, as well as human temporal lobe. The surface spikes were clustered and isolated; the origin of the spikes was verified in rats by simultaneously recording neurons with the movable tetrodes and silicon probes across all cortical layers. Numerous neurons, recorded from the superficial cortical layers, had a spatially unique distribution of action potential amplitude and morphology on the surface probe recording. These findings establish a method of recording individual neuronal firing activity from the surface of the brain. This new class of biocompatible, highly conformable devices allows for non-invasive recording of brain activity, enabling safe, reliable recording at the single unit level from surgical epilepsy patients.

Disclosures: D. Khodagholy: None. **J. Gelinas:** None. **W. Doyle:** None. **G. Malliaras:** None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

Title: High-density, high-speed electrical closed-loop system to control seizures

Authors: *Z. ZHAO, D. KHODAGHOLY, J. GELINAS, G. BUZSAKI
NYU Langone Med. Ctr., New York, NY

Abstract: Epilepsy is a major neurological disorder that affects almost 1% of population worldwide. Epileptic seizures are characterized by hypersynchronous neural firing within a neural network. Electrical stimulation may be effective in desynchronizing the firing pattern of these networks, interrupting the abnormal seizure discharges. To deliver real-time feedback electrical stimulation, a combination of recording system, controlling system and stimulation system is required. We have developed a closed-loop system as a platform for epilepsy studies. Our system consists of a high channel number time-division multiplexing system, with the ability

of switching between recording or stimulation state at any given time point. The stimulation profile is programmable on-chip, enabling the combination of different frequency components to build a variety of waveforms from simple sine waves to complex modulated waveforms. The system is based on compact and inexpensive commercial high performance chips, allowing for long-term closed-loop recording and stimulation in freely moving rodents. Here, we performed closed-loop intracranial recording and transcranial stimulation in rats. We were able to vary stimulation parameters (geometry of stimulating electrodes, frequency, waveform shape) to maximize entrainment of neural firing in superficial and deep brain structures. The system is tested to detect kindled hippocampal seizures and trigger responsive electrical stimulation to each abnormal discharge for demonstrating the feasibility of closed-loop transcranial electrical stimulation in suppression of synchronized epileptic discharges.

Disclosures: **Z. Zhao:** None. **D. Khodagholy:** None. **J. gelinas:** None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

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Title: State-dependent changes of hippocampal somatostatin interneuron activity and their effect on local circuit dynamics

Authors: *Y. SENZAI[1], G. BUZSAKI[2]
[1]New York Univ., New York, NY; [2]New York Univ., Neurosci. Inst., New York, NY

Abstract: The brain processes, stores, and retrieves relevant information from the environment for survival. In mammals, the hippocampus and interconnected structures have been shown to be important for this function and their activity patterns change according to different behavioral demands and brain states. It is recognized that interactions of various types of interneurons and excitatory neurons in the hippocampal cortex can affect their collective dynamics. However, it is poorly understood how different types of interneurons change their activity pattern across different brain states and how such changes affect the information processing done by them, such

as place field computation for navigation or memory. In order to address these questions, we employed mice expressing ChR2 or Arch selectively in somatostatin (SST)-positive neurons. SST neurons include some types of inhibitory neurons such as OLM cells, bistratified cells, and long range projecting inhibitory neurons. We examined large-scale recordings of LFP and units from the hippocampal subregions CA1 and dentate gyrus (DG), combined with optogenetical identification of SST neurons while the mouse performed on a linear track and sleeping in the home cage. We observed diversity of SST neurons in their firing patterns across different brain states. First, we classified state-dependent firing patterns of each hippocampal neuron subtype in CA1 and DG, combining optogenetical identification with physiological identification of fast spiking cells (putative baskets), non fast spiking cells, and pyramidal cells. Second, we also investigated the effect of SST neuron activation or inhibition on the local circuit dynamics, such as theta oscillation of LFP and local unit activities, across different brain states.

Disclosures: Y. Senzai: None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

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Title: Theta-gamma coupling in entorhinal-hippocampal networks

Authors: *E. W. SCHOMBURG[1], A. FERNANDEZ-RUIZ[1], K. MIZUSEKI[2], A. BERENYI[3], G. BUZSAKI[1]
[1]NYU Neurosci. Inst., New York, NY; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Dept. of Physiol., Univ. of Szeged, Szeged, Hungary

Abstract: The rodent hippocampus exhibits complicated but reproducible network activity patterns during exploratory behaviors, as well as during sleep. In addition to circuit-wide theta oscillations, gamma-band oscillations are spatiotemporally organized within the theta cycle in a brain state-dependent manner. The CA1 region of the hippocampus receives input from the CA3 region and layer 3 of the entorhinal cortex, and produces its own characteristic activity patterns.

Yet, the details and mechanisms of how the CA1 network integrates this input and coordinates its response *in vivo* are not well understood. We analyzed large-scale electrophysiological recordings from the dorsal hippocampus and medial entorhinal cortex in behaving and sleeping rats in order to characterize how local field potential (LFP) oscillations and unit spiking were coordinated within and between regions. We found that, while weak but significant LFP coherence could be detected up to the high-gamma frequency band (>80 Hz), putative pyramidal neuron spikes were timed largely independently of the gamma phases in the afferent regions. Rather, gamma-band power reflected the timing of inputs, with local interactions coordinating the fine timescale pyramidal cell responses. Furthermore, gamma coupling and the spike timing within the theta cycle varied with brain state, between waking and sleeping, as well as between recall and non-recall phases of a two-choice alternation task. Our results are consistent with a conceptual model in which CA1 integrates its two main excitatory inputs during the theta cycle, and that the relative strength of these inputs affects the dominant phase of CA1 output, but finer timescale coordination of this output is determined by interactions with the local interneuron network.

Disclosures: E.W. Schomburg: None. **A. Fernandez-Ruiz:** None. **A. Berenyi:** None. **K. Mizuseki:** None. **G. Buzsaki:** None.

Poster

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Support: HFSP LT-000346/2009-L

Machiah Foundation

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NIH NS34994

NIH MH54671

Title: Temporal precision of spiking in freely-moving animals

Authors: *E. STARK, D. ENGLISH, Y. SENZAI, L. ROUX, R. EICHLER, G. BUZSAKI
NYU Neurosci. Inst., New York, NY

Abstract: The nervous system represents internal processes and external events by spikes of single or multiple neurons. While the temporal precision of spike generation has been addressed before, it remains unknown how precise and reliable spike generation and transmission can be in freely-moving animals. We directly stimulated single cells or small groups of neighboring cells with a filtered white noise pattern (WN), while simultaneously recording population spiking and local field activity in the neocortex and hippocampus of awake-behaving rodents. We defined spiking reliability as the mean correlation between spike trains recorded during repeated stimulation trials. Precision was defined as the temporal jitter that yielded the same reliability as a spike-trigged average filter. Intra-cellular current injection into pyramidal cell (PYR) somata resulted in spike trains as reliable as 0.9 with 4 ms precision and 1 ms time lag. Yet overall, reliability and precision were lower than reported *in vitro*, and depended mainly on the injected current amplitude and baseline membrane potential variability. We then assessed the temporal precision of spiking generation using optogenetic depolarization of small neuronal groups using the same WN pattern. Individual directly-activated PYR exhibited low reliability and precision of spiking, consistent with a weak driving force and high baseline variability. Higher reliability and precision were observed for the agglomerated spike trains of multiple directly-activated PYR and for individual directly-activated parvalbumin-immunoreactive (PV) interneurons (0.5 with 3 ms precision and 1 ms time lag). Next, we examined reliability and precision in spike transmission by studying indirectly-activated post-synaptic cells. During direct PYR activation, individual indirectly-activated PV cells spiked with similar reliability and precision as directly-activated PV cells (0.5 with 3 ms precision). Notably, the time lag between the WN input and spiking was longer in indirectly-activated PV cells (4 ms), compared to directly-activated PV cells. During direct PV activation, PYR spiking was largely suppressed, yet remaining PYR spikes were as reliable as during direct PYR activation. Our results indicate that the serial combination of spike generation and transmission between PYR and PV in freely-moving animals is as precise as 3 ms and that introducing synaptic transmission does not decrease spiking precision for a given cell type. Thus, bi-directional spiking transmission between PYR and PV in freely-moving animals is at least as precise as the spike generating process.

Disclosures: E. Stark: None. **D. English:** None. **Y. Senzai:** None. **L. Roux:** None. **R. Eichler:** None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Machiah Foundation

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NIH NS34994

NIH MH54671

NSF (SMA1041755, TDLC)

Title: Pyramidal cell-interneuron interactions underlie hippocampal ripple oscillations

Authors: *L. ROUX[1], E. STARK[1], R. EICHLER[1], Y. SENZAI[1], S. ROYER[2], G. BUZSÁKI[1]
[1]NYU Med. Center, Neurosci. Inst., New York, NY; [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: High frequency ripple oscillations, observed in the hippocampal CA1 pyramidal layer during slow wave sleep, quiet wakefulness and consummatory behavior, are critical for memory consolidation. However, the cellular and network mechanisms underlying the generation, frequency control, and spatial coherence of the rhythm are poorly understood. To examine these mechanisms in the intact brain, we used optogenetic, pharmacological and closed-loop feedback tools in behaving and anesthetized mice and rats. We demonstrate that pyramidal neuron activity is a necessary requirement for ripple generation: depolarization of a small group of nearby pyramidal cells is sufficient to induce localized high frequency oscillations, and closed-loop silencing of pyramidal cells, or activation of parvalbumin or somatostatin positive interneurons, aborts spontaneously-occurring ripples. Moreover, focal pharmacological blockade of GABAA receptors abolishes ripples, indicating that fast inhibition is required for the generation of this oscillation. Finally, we found that localized PV interneuron activation paces ensemble spiking at ripple frequency, and that simultaneous induction of ripples at multiple locations results in a temporally coherent pattern mediated by phase-locked interneuron spiking. These observations show that inhibitory interactions play a critical role in ripple generation and synchronize independent rhythm generators. In conclusion, this work shows that temporally precise local interactions between excitatory and inhibitory neurons support ripple generation in the intact hippocampus.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

Title: An ethological profile of the interplay between sharp wave-ripples and theta activity in ca1

Authors: *J. D. LONG II, G. BUZSAKI
Neurosci. Inst., New York Univ., New York, NY

Abstract: The two-stage model of memory trace formation (Buzsáki 1989) postulates distinct behavioral correlates, physiological origins, and functional roles for hippocampal Sharp Wave-Ripples (SWRs) and theta activity. What has emerged is a picture in which theta activity is associated with exploration, information flow from the entorhinal cortex, and working memory, while SWRs are mapped to stationary behaviors, such as reward acquisition, and long-term memory. Recent work has borne out the relationship of SWRs to learning (Girardeau et al. 2009; Dupret et al. 2010) as well as the association between theta activity and both exploration and rapid modifications of place fields (Monaco et al. 2014). Here we provide a detailed study of the foraging behavior of the rat with the goal of expanding our understanding of the function of these neural features. We implanted high-density, linear silicon probes (6-8 shanks with 8-10 channels/shank, staggered vertically), bilaterally in dorsal CA1 of the rodent hippocampus (n = 4). These were positioned such that the channels of several shanks recorded neural activity across the pyramidal cell layer. By recording both the sharp wave and the ripple in both hemispheres simultaneously, we were able to robustly detect SWRs during behavior, which is often challenging using extracellular recording techniques, due to high-frequency artifacts produced during active behavior. In addition, our development of a markerless motion capture system provided us with a detailed record of our rodent subjects' kinematics in 3D as they foraged for reward. Together, these technologies allow us to tease apart the relationships between these neural and behavioral features, and to determine the strength of the associations between them. Of particular interest are the diverse scanning and orienting behaviors emitted when subjects search during exploration or discover, or hone in on, a reward location. To elicit these behaviors, we train rats to perform a foraging task, which requires learning a new reward configuration each session. A reward configuration is set at the beginning of each session, and

the subject must learn the reward locations within a 1.5 m diameter circular cheeseboard maze. Our observation of bouts of SWRs as our subjects orient around reward locations, suggests a delicate interplay between the limits of working memory, expressed through theta activity, and the need to encode this information before it is lost, via SWRs.

Disclosures: J.D. Long II: None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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NIH Grant R01NS034994-16

APA-Lilly Psychiatric Research Fellowship

Title: Changes in neocortical circuit dynamics during sleep

Authors: *B. O. WATSON[1,2], J. P. GREENE, II[1], G. BUZSAKI[1]
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Abstract: Sleep is a poorly understood phenomenon, despite the large proportion of mammalian life consumed by this activity. Over the years a large number of experiments have been conducted to attempt to elucidate the function of sleep, often functioning on learning and memory. More recent theories have related sleep to homeostatic mechanisms, putatively including re-scaling of neuronal firing rates and synapses. Studies have demonstrated that over the course of sleep, neocortical spike rates decrease while they rise after waking (Vyazovsky 2009). More recent findings give evidence that in hippocampal circuits, Rapid Eye Movement (REM) sleep may be primarily responsible for the shift in spike rates over the course of sleep (Grosmark, 2012). It remains to be determined whether the role of REM and non-REM in rescaling neural spike rates is the same in the neocortex, or whether Slow Wave Sleep (SWS) is more responsible, as has been implied. We are using silicon probes to perform high-density recording in neocortical circuits in rats in order to address this question. We examine relative effects in excitatory versus inhibitory populations and find, consistent with prior results, that

spike rates are increased during REM sleep in cortical populations but that in at least a fraction of neurons the net effect of REM is to decrease spike rate. Importantly, we also examine short-time scale interactions between pairs of neurons as an indirect measure of synaptic strength. We find that over the course of sleep, putative connections between principal cells and interneurons undergo significant changes so that the overall coefficient of variation of synaptic strength decreases. 1. Cortical firing and sleep homeostasis. Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G. Neuron. 2009 Sep 24;63(6):865-78. 2. REM sleep reorganizes hippocampal excitability. Grosmark AD, Mizuseki K, Pastalkova E, Diba K, Buzsáki G. Neuron. 2012 Sep 20;75(6):1001-7.

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Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Title: Intracellular correlates of sharp-wave ripple network events in CA1 pyramidal neurons

Authors: *D. F. ENGLISH, A. PEYRACHE, E. STARK, L. ROUX, D. VALLENTIN, M. A. LONG, G. BUZSAKI
Neurosci., The Neurosci. Institute, New York University, Sch. of Med., New York, NY

Abstract: Sharp-wave associated ripple events (SWR) in the CA1 region of the hippocampus are considered to be an essential component of the repertoire of oscillatory functions the hippocampus utilizes to process information and share it with the neocortex. Sharp waves are generated by a massive excitation of CA1 pyramidal neurons by the CA3 region, while the excitation of CA1 interneurons interacting with the depolarized CA1 pyramidal neurons generates the ~150 Hz ripple event observed in the pyramidal layer LFP and in the membrane

potential (Vm) of CA1 pyramidal neurons. A specific importance has been placed on the information content (in the form of pyramidal neuron action potentials) of SWR events, due to observations that their suppression impairs spatial memory, that ensembles of CA1 pyramidal neurons active in a specific sequence during spatial exploration are replayed during subsequent SWR events at compressed timescales and that SWRs massively recruit cortical and subcortical targets, a phenomenon thought to underlie systems level memory consolidation. In a given SWR event ≈10% of CA1 pyramidal neurons contribute an action potential. To better understand the role of CA1 pyramidal neuron membrane dynamics and synaptic inputs in ripple generation and in determining their sparse but crucially important participation in SWR events, we used a recently developed method to record the Vm of CA1 pyramidal neurons *in vivo* in freely moving/sleeping mice. We additionally recorded SWRs in the extracellular LFP from the ipsilateral CA1 pyramidal layer. Our data demonstrate a precise temporal relationship between the Vm of pyramidal neurons and the LFP recorded in the pyramidal layer during SWRs, wherein pyramidal neuron Vm oscillates at LFP ripple frequency and in coherence with the LFP ripple. Additionally, our data support previous evidence suggesting that pyramidal neurons experience exceptionally strong inhibition during ripples. Lastly, our data show that action potentials are generated orthodromically in naturally occurring ripples *in vivo*, ruling out the possibility of an axonic origin of ripple generation. In conclusion, our data support a model of ripple generation in which an excitatory barrage from CA3 impinging on the CA1 network systematically depolarizes a large portion of the CA1 pyramidal cells while perisomatic inhibition functions to control the generation and timing of action potentials.

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Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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NIH Grant R01NS034994-16

Title: Hippocampal interictal discharges and sleep spindles: Interference with physiological consolidation?

Authors: *J. GELINAS, D. KHODAGHOLY, G. BUZSAKI
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Abstract: Mesial temporal lobe epilepsy continues to be one of the most common pharmacoresistant focal epilepsy syndromes, and is associated with deteriorating cognitive function if seizures persist. In addition, hippocampal interictal epileptiform discharges (IEDs) transiently disrupt cognitive processes in animal models and humans. However, the specific neuronal networks and mechanisms that underlie this disruption remain unclear. Temporal coupling between hippocampal sharp-wave/ripple (SWR) complexes and spindles in the medial prefrontal cortex (mPFC) is thought to play a role in memory consolidation during sleep. We hypothesized that hippocampal IEDs are similarly able to interact with mPFC, and may interfere with physiological cortico-hippocampal coupling. Here, local field potentials and multiunit activity were recorded from medial prefrontal cortex and hippocampus over the course of commissural pathway kindling in rats. Event correlation of spindle activity in the mPFC and SWR or IED complexes in the hippocampus was calculated. As kindling progresses, spindle-like activity becomes evident within 500 ms of hippocampal IEDs. IED-associated spindles resemble physiologic spindles in frequency and duration. Frequent occurrence of hippocampal IEDs is also associated with fewer SWRs. These results suggest that hippocampal IEDs and SWRs may compete for influence in hippocampal-targeted cortical regions. Interictal hippocampal network activity could disrupt physiologic oscillations critical for memory consolidation, providing a possible mechanism for cognitive impairment in epilepsy.

Disclosures: J. Gelinas: None. **D. Khodagholy:** None. **G. Buzsaki:** None.

Poster

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Title: Self-organized mechanisms of the head direction sense

Authors: *A. PEYRACHE[1], P. PETERSEN[2], M. LACROIX[2], G. BUZSAKI[2]
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Abstract: The relationship between stimulus-driven and self-generated ('spontaneous') activity is a recurring topic in neuroscience. We chose the head direction (HD) system to address this problem because of its simplicity. The HD system may form a labeled line system in which feed-forwardly connected subcortical, thalamic and cortical HD neurons are driven by vestibular inputs. Alternatively, the inputs may impinge upon preconfigured networks, whose activity can enhance the precision of the peripheral sensors. To choose between those frameworks, we examined ensembles of HD neurons in the anterodorsal thalamic nucleus (ADN) and in its main cortical target, the post-subiculum (PoS), in mice during waking and sleep. In the ADN, activity of HD neuron pairs with <60° orientation preference were positively correlated at both fast (5-10 ms) and slow (>100 ms) time scales in all brain states. These slow correlations were preserved between the HD cells of the two brain regions across brain states, indicating that the internal HD signals remained in register at all times. The fast thalamic coordination is shown to very strongly recruit cortical cells, thus providing a physiological mechanism enabling an efficient and reliable information transmission. Finally, the behavior of the animals could not account fully for the amount of slow correlations in the ADN: they were stronger (or weaker) than predicted for cell pairs closer (or further) than 60° apart. Taken together, these results provide evidence for self-organized mechanisms amplifying head direction information.

Disclosures: **A. Peyrache:** None. **P. Petersen:** None. **M. Lacroix:** None. **G. Buzsaki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 466.22/UU58

Topic: F.02. Animal Cognition and Behavior

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Title: Inhibition-based theta resonance in a hippocampal network: A modeling study

Authors: *H. G. ROTSTEIN[1], E. STARK[2], G. BUZSÁKI[2]
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Abstract: Network rhythmic oscillations result from the cooperative activity of the participating neurons and synaptic connectivity. Several neuron types exhibit subthreshold (membrane potential) resonance (a peak in the voltage amplitude response to oscillatory current inputs at a preferred, resonant, frequency). Whether and how subthreshold preferred frequency responses translate to the spiking regimes in single cells and networks (spiking and network resonance) are still open questions. We use mathematical modeling and simulations to address these issues in the context of *in vivo* experimental results where pyramidal cells (PYR) and parvalbumin-immunoreative interneurons (INT) were optogenetically stimulated using wide-band (WB) oscillatory signals (Stark et al., Neuron, 2013). Dependence of spiking activity on input frequency was measured by the spectral coherence between the input and output signals. While PYR have been shown to exhibit theta subthreshold resonance *in vitro* (Hu et al., J Physiol, 2002), *in vivo* responses of individual directly stimulated PYR were not predominantly at theta, but WB as INT were. In contrast, PYR exhibited theta band-limited rebound spiking induced through direct stimulation of INT, which exhibited a WB response. We present a minimal biophysical (conductance-based) model of a CA1 hippocampal network that captures these experimental results. The basic model includes PYR and INT. The extended model includes also OLM (orients-lacunosum moleculare cells). PYR and OLM included h-currents. The presence of subthreshold resonance in isolated PYR is not communicated to the spiking regime mainly due to the strong effect of the oscillatory input amplitude. PYR theta-band response results from a combination of rebound spiking and a timing mechanism. Rebound spiking is responsible for the generation of spikes at input frequencies that are low enough for the voltage response to be above threshold. The timing mechanisms are responsible for "erasing" spikes generated by input frequencies lower thant theta. We implemented two such mechanisms: (i) network-mediated inhibition from OLM or (ii) synaptic depression of INT synapses. Overall, these results provide a mechanistic understanding of network resonance at theta frequencies.

Disclosures: H.G. Rotstein: None. **E. Stark:** None. **G. Buzsáki:** None.

Poster

466. Cortical and Hippocampal Circuits: Timing and Temporal Processing I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH054671-14

NIH Grant R01NS034994-16

APA-Lilly Psychiatric Research Fellowship

Title: Electrophyisiologic characterization of medial prefrontal cortical response to ketamine

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Abstract: Ketamine has long been known as a dissociative anesthetic. In the last decade or so, it has been found, however, to be a highly effective antidepressant when given in lower doses. Clinical data is particularly compelling since it demonstrates that a single injection of ketamine can have antidepressant effects starting within hours and lasting for days and even has high efficacy rates in suicidal and treatment resistant patients. This stands in contrast to traditional antidepressants that can take weeks to work and seem to work in fewer patients and therefore opens the scientific question of what ketamine can teach us about the neurobiology of depression. The fast time scale and long-lasting nature of the response to ketamine stands in contrast to the traditional view that depression can take weeks to properly treat. Over the last few years, studies have been carried out to assess the molecular, cell-biologic (Li 2011) and even neuromodulator-based effects of ketamine. Of particular interest to us are findings suggesting synaptogenesis in the medial prefrontal 24 hours after injection of antidepressant-dose ketamine in mice. This synaptogenesis was observed via imaging of dendritic spines, but it has not yet been established whether the newly generated synapses are active or otherwise functionally relevant to the microcircuit. Here we use silicon probes to perform high-density electrophysiologic recordings in the medial prefrontal cortex of rats to characterize the response of prefrontal networks to intraperitoneal ketamine injection. We assess a number of aspects of network response at various time points from a long 6-hour baseline period to a full 24 hours post-injection. We measure spike rate changes across cell populations, local field potential changes, sleep behavior changes, and activity at sites of functional connection between individual units. Our recordings span a full 24 hours post-injection in order to 1) characterize changes concurrent with the synaptic changes documented in the past and 2) in order to control

for regular circadian variations in neurophysiology. 1. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS. Science. 2010 Aug 20;329(5994):959-64.

Disclosures: **J.P. Greene:** A. Employment/Salary (full or part-time):; Salary partially paid by American Psychiatric Association-Lilly Research Fellowship. **B.O. Watson:** Other; Research partially paid by American Psychiatric Association-Lilly Research Fellowship. **G. Buzsaki:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: INSERM

Institut de Recherche, Servier

Title: Acute tianeptine treatment selectively modulates neuronal activation in the central nucleus of the amygdala and attenuates fear extinction

Authors: *B. P. GODSIL[1,2], B. BONTEMPI[3], F. MAILLIET[1], P. DELAGRANGE[4], M. SPEDDING[5], T. M. JAY[1,2]
[1]INSERM U894, Paris, France; [2]Univ. Paris Descartes, Paris, France; [3]Inst. des Maladies Neurodégénératives, CNRS UMR 5293 and Universités Bordeaux 1 et 2, Talence, France; [4]Inst. de Recherche, Servier, Suresnes, France; [5]Spedding Res. Solutions, Le Vesinet, France

Abstract: Exposure based therapies (EBT) and antidepressants are both commonly prescribed treatments for anxiety disorders and there is growing interest in understanding how antidepressants might impact EBT. Tianeptine is an antidepressant with anxiolytic effects and it produces unique effects on stress via glutamatergic systems in limbic regions. Chronic tianeptine treatment has been shown to reduce the acquisition of amygdala-based extinction learning, whereas the drug's acute influence on neural activation in limbic regions, and on extinction learning, is not well understood. To assess its acute influence on neuronal activation rats were injected with tianeptine and FOS immunoreactivity was measured in amygdala and prefrontal

cortex subregions. To test its acute influence on extinction processes, rats were given tianeptine before contextual and auditory fear extinction training procedures, and before an extinction expression test. Acute tianeptine treatment selectively modulated FOS expression in the central nucleus of the amygdala (CE) depending on ongoing activation. In conditions involving high background expression, tianeptine decreased FOS levels in the CE, whereas tianeptine induced FOS increases in low background conditions. Acute tianeptine also attenuated the extinction of fear, as well as the recall of fear extinction memory. The CE has an established role in emotional processing related to fear, as well as habit formation and reinforcement. Thus selective modulation of the CE might be pivotal for elucidating the mechanism supporting tianeptine's clinical efficacy. These results also underscore that acute tianeptine dosing could alter extinction learning during EBT, possibly due to the drug's interaction with stress.

Disclosures: **B.P. Godsil:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Institut de Recherche, Servier provided grant and material support for this project.. **B. Bontempi:** None. **F. Mailliet:** None. **P. Delagrange:** A. Employment/Salary (full or part-time):; Institut de Recherche, Servier. **M. Spedding:** A. Employment/Salary (full or part-time):; Formerly employed the IRS. **T.M. Jay:** 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.; Received grants from IRS.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NS034007

NS047384

Title: Differential translational control mechanisms mediating the persistence of memory

Authors: *T. N. HUYNH, E. SANTINI, E. KLANN
Ctr. for Neural Science, New York Univ., New York, NY

Abstract: De novo protein synthesis is required for the reconsolidation and extinction of cued fear memory. Central to the regulation of translation is the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, which stimulates cap-dependent translation through the phosphorylation of two downstream effectors: eIF4E-binding proteins (4E-BPs) and p70 S6 kinase 1 (S6K1). Phosphorylation of 4E-BPs permits the binding of eIF4E to eIF4G to form eIF4F, and phosphorylation of S6K1 leads to the phosphorylation of additional translational control molecules, to stimulate protein synthesis. Previous reports indicate that rapamycin, an inhibitor of mTORC1, blocks the reconsolidation of cued fear memory. Therefore, we determined the specific roles of the mTORC1 effectors in reconsolidation. Consistent with previous reports, we found that inhibition of eIF4E-eIF4G interactions with 4EGI-1 was not sufficient to block reconsolidation. We also found that inhibition of S6K1 with PF-4708671, did not block reconsolidation. However, inhibiting both eIF4E-eIF4G interactions and S6K1 blocked the reconsolidation of cued fear memory. These findings indicate that concomitant activation of two mTORC1 effectors, eIF4F and S6K1, is required for memory reconsolidation. We proceeded to determine the role of mTORC1 in the extinction of cued fear memory. We found that rapamycin had no effect on the acquisition of extinction, but blocked extinction memory. Similarly, inhibition of eIF4E-eIF4G interactions blocked the consolidation of extinction. We then examined the role of S6K1 in extinction. We found that both pharmacological inhibition and genetic ablation of S6K1 impaired acquisition of extinction. In addition, our preliminary biochemical studies indicate that S6K1 is regulated by extracellular signal-regulated kinase, but not mTORC1, during acquisition of extinction. Overall, the results of our studies are consistent with a role for mTORC1 and its downstream effectors in the reconsolidation and extinction of conditioned fear memory.

Disclosures: **T.N. Huynh:** None. **E. Santini:** None. **E. Klann:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 5R01NS080388-03

Title: Nogo Receptor 1 limits fear extinction learning in adulthood

Authors: *S. M. BHAGAT[1], S. M. STRITTMATTER[2]
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Abstract: In adult rodents, fear conditioning produces a lifelong fear memory that cannot be permanently repressed or erased, even following extinction training. This contrasts with what has been reported in juvenile rodents, which exhibit fear erasure following extinction training. The formation of myelin and perineuronal nets, which are extracellular matrices rich of chondroitin sulfate proteoglycans, coincide with the critical period for fear erasure. We have found that Nogo Receptor 1 (NgR1), a neuronal receptor for myelin associated inhibitors that block neuronal sprouting and cortical plasticity in adulthood, significantly inhibits plasticity during fear and extinction learning. We have found that adult male NgR1 knockout (KO) mice, in contrast to wild-type (WT) controls, show a significant enhancement in fear extinction recall and no spontaneous recovery, a behavioral assay to measure recall of the original fear memory. We have used conditional knockouts to explore the temporal and regional role of NgR1. In conclusion, NgR1 plays a pivotal role in limiting extinction learning in adulthood, which could help enhance our current insight into neuropsychiatric diseases, such as anxiety disorders and post-traumatic stress disorder (PTSD).

Disclosures: S.M. Bhagat: None. **S.M. Strittmatter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of Axerion Therapeutics seeking to develop PrP and NgR therapies..

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Program#/Poster#: 467.04/UU63

Topic: F.02. Animal Cognition and Behavior

Support: NSFC 81201032

NSFC 81221002

Title: Effect of food restriction on extinction, recovery and persistence of auditory fear memory in rats

Authors: *P. WU, S.-Q. MENG, Y.-X. XUE, L. LU
Natl. Inst. On Drug Dependence, Peking Univ., Beijing, China

Abstract: Background: As demonstrated by previous studies, food restriction (FR) can protect neurons against degeneration in animal models of Alzheimer's, Parkinson's and Huntington's diseases and stroke. It also could stimulate the production of new neurons from stem cells (neurogenesis) and can enhance synaptic plasticity, which may increase the ability of learning and memory. Recently, a short period of food restriction in adulthood was shown to restore plasticity in visual cortex. Thus, we hypothesized that food restriction might influence the extinction, recovery and persistence of fear memory by re-juvenile the synaptic plasticity. Methods: The rats were trained for auditory fear memory, followed by a period of 28 days food restriction, during which rats were access to food only on alternate days. After that, a group of rats were tested for persistence of auditory fear memory, and three groups of rats underwent memory extinction and test for reinstatement, spontaneous recovery, and renewal of auditory fear memory. Results: Compared with the rats on normal dietary regiment, the rats in food restriction group showed accelerated extinction, decreased return in reinstatement, spontaneous recovery and renewal test. Food restriction also attenuated the persistence of fear memory and reduced anxiety performance in elevated plus maze and open field test. Conclusion: Food restriction in fear conditioned rats promoted extinction and reduced the return of extinguished fear might by restoring the plasticity in the extinction-related circuits.

Disclosures: **P. Wu:** None. **S. Meng:** None. **Y. Xue:** None. **L. Lu:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP 2011/22523-0

Title: Impaired extinction of contextual fear conditioning in iNOS knockout mice is associated to changes in mRNA expression of nitric oxide and endocannabinoid system components

Authors: ***S. F. LISBOA**[1,2], F. V. GOMES[1,2], A. L. DA SILVA[1], F. Q. CUNHA[1], S. R. L. JOCA[3,2], F. S. GUIMARÃES[1,2], L. B. M. RESSTEL[1,2]
[1]Pharmacol., Univ. of São Paulo - FMRP, Ribeirão Preto, Brazil; [2]Ctr. for Interdisciplinary Res. on Applied Neurosciences (NAPNA), São Paulo, Brazil; [3]Pharmacol., Univ. of São Paulo - FCFRP, Ribeirão Preto, Brazil

Abstract: Atypical neurotransmitters such as nitric oxide (NO) and endocannabinoids (ECBs) modulate defensive behavior. Inducible NO synthase enzyme (iNOS) and cannabinoid receptor type 1(CB1) gene deletions increase anxiety-like behavior in mice, whereas deletion of the neuronal NOS isoform (nNOS) is anxiolytic. iNOS knockout mice (iNOS KO) present increased contextual fear conditioning (CFC) expression and impaired fear extinction associated with increased basal NOS activity in the medial prefrontal cortex (MPFC). NOS inhibitors prevent these changes. In the present work we investigated if these changes depend on compensatory increase of basal nNOS activity and/or changes in the ECB system. Behavioral experiments with iNOS KO and wild-type (WT) mice were performed on four consecutive days, with CFC taking place on day 1 (3 footshocks, 0.75 mA, 1 s each) and extinction evaluation 24h, 48h, 72h and 96h later. mRNA expression of nitrergic (nNOS and eNOS) and ECB system components (CB1, CB2, fatty acid amide hydrolase-FAAH and monoacilglicerol lipase - MAG) was measured by qPCR in the MPFC and hippocampus (HIP) 24h after conditioning. CFC expression was similar in WT and KO mice, but the latter showed extinction deficits ($p<0.05$, repeated measures ANOVA). Several changes in mRNA expression of NOS enzymes and ECB-related genes were observed. In the MPFC, KO mice showed increased and decreased expression of CB1 and CB2 receptors in naive and after fear conditioning ($p<0.05$, Student t test), respectively. Opposite results were found with the metabolizing enzymes (FAAH and MAGL) ($p<0.05$, Student t test). On the contrary, fear conditioning increased nNOS and eNOS mRNA expression in KO mice ($p<0.05$, Student t test). In the HIP, non-conditioned KO mice presented decrease expression of CB1 and CB2 receptors ($p<0.05$, Student t test). There was, however, no difference after fear conditioning between iNOS KO and WT mice. In addition, conditioning also decreased mRNA expression of CB2 and increased of FAAH and MAGL in WT mice ($p<0.05$) and increased of eNOS in KO mice ($p<0.05$). These results suggest that extinction deficits in CFC could be related to dysregulation of the endocannabinoid system *in situ*ations where NO signaling is increased, such as in iNOS KO mice. These mice could be a suitable genetic model to investigate disorders with impaired extinction, such as PTSD. In addition, atypical neurotransmitters such as NO and endocannabinoids could be a therapeutic targeted in these disorders.

Disclosures: S.F. Lisboa: None. **F.V. Gomes:** None. **A.L. da Silva:** None. **F.Q. Cunha:** None. **S.R.L. Joca:** None. **F.S. Guimarães:** None. **L.B.M. Resstel:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

CNPq

CAPES

FAEPA

Title: Dorsolateral periaqueductal gray matter endocannabinoid system modulates the expression of contextual fear conditioning: Involvement of local nmda receptors, nitric oxide and trpv1 receptors

Authors: *L. B. RESSTEL, D. ULIANA, S. HOTT, S. LISBOA
Pharmacol, Schl Med, FMRP-USP, Ribeirao Preto- SP, Brazil

Abstract: The dorsolateral periaqueductal gray matter (dlPAG) endocannabinoid system has been related modulate the expression of contextual fear conditioning (CFC). This system is able to modulate the glutamatergic system in the dlPAG by presynaptic CB1 receptors decreasing the glutamate releases. Moreover, it was described that dlPAG glutamatergic NMDA receptors and the nitric oxide (NO) synthesis interaction is involved whit modulation of CER. The dlPAG TRPV1 receptors are activated by endocannabinoid system and they are involved with defensive reactions. Therefore, the aim of this work was to verify if CER modulation by dlPAG CB1 receptors involves NMDA receptors, NO and TRPV1 receptors. Male Wistar rats (240-260g) with unilaterally implanted guide cannula aimed at the dlPAG were first exposed to a box during 10 min for habituation and in a second exposure to the same box; they received 3 electrical footshocks (0.85 mA, 2 s). One day later, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. After additional 24h the behavioral and autonomic responses (mean arterial pressure - MAP, heart rate - HR and cutaneous temperature - CT) were measured in a 10 min test session during re-exposition to the same context, but without delivery of shocks. Vehicle (saline or DMSO 10%; 0.1 μL) or/and the CB1 antagonist (AM251 0.3 nmol) were administrated in dlPAG. Five minutes after the first injection, NMDA antagonist (AP7; 1nmol); nNOS inhibitor (N-Propyl; 0.4 nmol) or TRPV1 antagonist (6-iodonordihydrocapsaicin , 6-iodo, 3nmol) were administered in the dlPAG 10 min before the test. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 127/2011). The AM251 in the doses of 0.3 nmol (n=8) increased the percentage of freezing ($t=3.5$, $P<0.001$), and also inducing an increase the rise MAP ($F_{1,182}=21.4$, $P< 0.01$) and HR ($F_{1,182}=25.8$, $P<0.001$) and the decrease of CT ($F_{1,182}=18.21$, $P<0.001$). The NMDA antagonism by AP7 (n=8) the effects of AM251 on freezing ($F_{3,27}=7.39$, $P<0.001$), MAP ($F_{3,378}=9.46$, $P<0.001$), HR ($F_{3,378}=7.72$, $P < 0.0001$) and CT ($F_{3,378}=5.19$, $P<0.001$). Similar was observed with the N-propyl (n=7) (Freezing: $F_{3,28}=50.87$, $P<0.001$; MAP: $F_{3,392}=23.44$, $P<0.001$, HR: $F_{3,392}=17.53$, $P<0.001$, CT: $F_{3,392}=17.49$, $P< 0.0001$) and the 6-

iodo (n=7) (Freezing: $F_{3,23} = 11.41$, $P<0.001$; MAP: $F_{3,322}=12.35$, $P<0.001$; HR: $F_{3,322}=23.71$, $P<0.001$; CT: $F_{3,322}=20.32$, $P<0.001$). Our findings showed that the modulation of dlPAG endocannabinoid system on the contextual fear conditioning involves NMDA receptors, NO synthesis and TRPV1 receptors.

Disclosures: **L.B. Resstel:** None. **D. Uliana:** None. **S. Hott:** None. **S. Lisboa:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

Support: St. Mary's College of Maryland

Title: D-cycloserine hastens extinction of fear conditioning in a latent inhibition paradigm but does not interact with prolonged extinction in the pre-exposed groups

Authors: ***W. P. JORDAN**, S. A. D'AMBROSIO, N. E. DEANGELI, M. L. KLIMA, H. J. PRIBUT
St Mary's Col. of MD, St. Marys City, MD

Abstract: d-cycloserine (DCS) enhances extinction of conditioned fear in rats and is useful clinically in the treatment of phobias. DCS's effects on extinction have not been studied in a latent inhibition (LI) paradigm in which some animals receive pre-exposure to the to-be conditioned stimulus (CS) prior to fear conditioning and extinction. Jordan and Leaton (2013) found that rats pre-exposed to a noise CS and then conditioned with footshock were slower to extinguish the fear response compared to controls. This "post-LI extinction" effect has been replicated, but a theoretical explanation is elusive. The current study asked whether DCS would modify or abolish this effect. 39 adult, male Sprague-Dawley rats were water deprived and trained to lick. Group PE was given two 10-min pre-exposure sessions of 10 noise stimuli (88dB, 20s) per day on a 40 to 60-s ISI while licking. Group NPE received a single stimulus presentation to reduce unconditioned suppression to the CS in conditioning. Four days of fear conditioning (C) with one CS/US (0.5mA, 0.5s footshock) per day, was followed by 9 extinction (E) sessions with multiple CS presentations. 30-min prior to the first E session, half of each group was injected intraperitonealy with 30 mg/kg of DCS. Control animals received saline. Both the PE and NPE groups habituated lick suppression to the CS during PE sessions.

Acquisition of fear was significantly retarded in the PE Group, showing LI, but both groups reached full suppression by the end of conditioning. Responsiveness on E1 was not affected by DCS. Extinction was slow to develop in all groups, suggesting that DCS administration did not immediately weaken the fear response. As testing continued, both PE Groups extinguished more slowly than the NPE Groups, the difference reaching significance by E9, replicating the post-LI extinction effect of Jordan & Leaton (2013). DCS-treated animals extinguished more than vehicle controls in both the PE and NPE groups. This enhancement of extinction was equivalent in both groups--there was no significant DCS x PE interaction. Thus, DCS did not alter post-LI extinction other than to superimpose its effect on that of the effect of pre-exposure. These results confirm that whatever happened in PE to produce LI survived subsequent asymptotic conditioning to prolong extinction. These lasting effects of PE are not altered by DCS. Whatever brain mechanisms support the post-LI extinction effect are not sensitive to activation of the strychnine-insensitive glycine site of NMDA glutamate receptors (the target of DCS). The neural substrates for the post-LI extinction effect must be sought elsewhere.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Program#/Poster#: 467.08/UU67

Topic: F.02. Animal Cognition and Behavior

Title: Extinction of spatial navigation in the Morris water task: The effect of brief reminders

Authors: *T. DONALDSON[1], C. MAGCALAS[1], D. BARTO[1], K. AKERS[2], D. HAMILTON[1]
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Abstract: Spatial navigation is essential to living organisms. The Morris water task (MWT) has been used extensively to investigate the spatial navigation abilities of rats and other rodents in the laboratory. Numerous studies have examined the processes involved in spatial learning and memory using the MWT, however, comparatively little is known about extinction of spatial responding or the factors that influence extinction. In a typical probe trial with the escape platform removed from the pool rats will persist in searching at the platform location, particularly earlier during the probe trial. Here we investigated extinction of spatial responding when the platform is removed during multiple no-platform probe trials, and the influence that

brief reminder treatments, in the form of platform placement, have on the rate of extinction of spatial responding. Extinction of spatial responding will be characterized by decreased accuracy in navigation and searching behavior across successive probe trials. Adult male Long-Evans rats were given either 2 or 4 days of training (12 trials per day). One day after completion of training the rats received either a brief reminder treatment (30 sec placement on the platform in the trained location) or no treatment followed by two 60 sec no-platform probe trials separated by 1 min. Latency and path length to enter and time and distance spent within a critical region (50cm diameter, centered on the platform location) were measured for each probe trial. Rats in the no reminder group (no platform placement) exhibited increased latency and path length to enter the critical region while rats in the reminder condition (platform placement) did not display a robust decline in these measures across the two probe trials. The reduction in extinction following the reminder treatment for these measures was observed following 2 and 4 days of training. No effects of the reminder treatment on extinction were observed for measures related to persistence in responding. These findings suggest that brief placement on the platform makes spatial navigation to the escape platform in the MWT more resistant to extinction. Further, while platform placement has been shown to improve some aspects of performance during subsequent training, the available literature provides data that are mixed, weak, or fail to support the idea that platform placement in the absence of active swimming to the platform can result in unambiguous spatial navigation in the MWT. The present findings establish that post-training platform placement can, however, clearly reduce the rate of extinction during subsequent no-platform probe trials.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Program#/Poster#: 467.09/UU68

Topic: F.02. Animal Cognition and Behavior

Support: George Mason University Office of Student Scholarship, Creative Activities, and Research

Title: The effects of mild copper deficiency on fear extinction and motor behavior

Authors: *C. NEELY, S. LIPPI, S. WILKINS, J. FLINN
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Abstract: Zinc (Zn) and Copper (Cu) are essential biometals which are incorporated in enzymes and serve as catalysts throughout multiple bodily systems. Zn supplementation prevents adequate absorption of Cu through the intestinal wall. Zinc-induced deficits in spatial memory and the extinction of learned fear were remediated through Cu supplementation (Railey et al., 2010). Thus the effects of excess Zn may be due to a deficiency in Cu (Maret & Sandstead, 2006). To explore the effects of Zn/Cu ratio alterations, a copper deficient diet and specialized control diet have been developed, in association with Harlan, with Zn levels remaining constant (approximately 40ppm Zn) and the Cu levels differing between the Cu deficient diet (4ppm) and Cu control diet (16 ppm). Forty-five Sprague Dawley rats underwent fear conditioning, extinction, and accelerating rotarod testing in 3 groups: prenatal Cu deficient, postnatal Cu deficient and postnatal Cu control. Surprisingly, all groups showed weaker fear extinction than previously seen in rats raised on a 7012 control diet; however, these differences did not reach significance. The prenatal Cu deficient rats and postnatal Cu control rats showed differences in freezing on the second day of fear extinction recall (FER), $F(2, 42) = 2.51$, $p = 0.09$, with the prenatal Cu deficient group having higher levels of freezing. Follow-up analyses for FER day 2 on trial 9 revealed the largest difference in freezing levels, $F(2, 42) = 9.15$, $p < 0.01$ between the prenatal Cu deficient group ($M = 52\%$) and postnatal Cu deficient ($M = 27\%$) and postnatal Cu control ($M = 17\%$) dietary conditions. For the accelerating rotarod, repeated measures ANOVA revealed significant differences across all trials, $F(2, 35) = 3.63$, $p < 0.05$, and demonstrated that the prenatal Cu deficient rats had significantly greater motor impairment than the postnatal Cu deficient rats. In summary, rats on the postnatal Cu control diet condition did not show as rapid fear extinction as expected. We suggest that ingredients in the control diets, e.g. fat content and soy, had behavioral effects and we are assessing these potential confounds in current studies.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

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Title: Neuronal activity in prelimbic cortex correlates with expression of active avoidance

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Abstract: We recently reported that muscimol inactivation of the prelimbic prefrontal cortex (PL) impairs platform-mediated avoidance, in which rats avoid a tone-signaled footshock by stepping onto a nearby platform (Bravo-Rivera et al., 2014). In the current study, we used single-unit recording to determine if PL neurons are responsive to the tone, platform approach, or both, in rats trained in platform-mediated avoidance. A total of 440 PL neurons were analyzed. Neurons were classified as tone-responsive (TR cells) if their firing rate in the first 3-second bin following tone onset differed significantly from baseline ($-2.58 > Z > 2.58$). We found that 23% of PL neurons exhibited tone responses, which is comparable to fear conditioning (25%, Burgos-Robles et al., 2009; Sotres-Bayon et al., 2012). Interestingly, avoidance was associated with a higher percentage of inhibitory TR cells than fear conditioning (19% vs. 5%). We next examined neural correlates of platform approach, which typically occurred within 10s of tone onset, and to a lesser extent in the absence of the tone. PL neurons were classified as approach responsive if their firing rate increased or decreased within 3 seconds prior to platform mounting ($-3.00 > Z > 3.00$). Using this criterion, 38% of PL neurons signaled platform approach, which was significantly higher than in an unconditioned control group (13%, Fisher Exact, $p<0.05$), suggesting that PL activity reflects avoidance behavior. Cells responded to platform only (15%), tone only (8%), or both (23%). Platform approach responses were more often excitatory than inhibitory (39% vs. 16%, $p<0.01$). Most cells that responded to the tone also responded to the approach (63%), and vice versa (61%). Together, our findings suggest that inhibitory tone responses in PL emerge with avoidance training and that inputs signaling the tone converge with inputs signaling platform approach. Future studies using an optogenetic approach will determine which of these PL correlates is necessary for expression platform-mediated avoidance.

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Poster

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Title: An avoidance-based model of exposure with response prevention in rats

Authors: *J. RODRIGUEZ-ROMAGUERA[1,2], H. BRAVO-RIVERA[1], E. I. GONZÁLEZ-ARAYA[1], A. M. MINIER-TORIBO[1], B. D. GREENBERG[3], S. A. RASMUSSEN[3], G. J. QUIRK[1]
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Abstract: Patients suffering from the harm-avoidant type of obsessive-compulsive disorder (OCD) believe their compulsions protect them from danger (Rasmussen, 1992). Compulsions therefore can be viewed as persistent avoidance responses. OCD is treated with exposure-with-response-prevention (ERP) therapy, in which patients are repeatedly exposed to trigger stimuli but prevented from expressing their compulsions. ERP is thought to be based on extinction of these avoidant-type responses, but mechanistic studies await a suitable rat model. We developed an extinction-with-response prevention ("Ext-RP") task, using a novel platform-mediated avoidance task in which rats avoid a tone-signaled shock by stepping onto a nearby platform (Bravo-Rivera et al., 2014). Following avoidance training, access to the platform is blocked with a Plexiglas barrier, and rats undergo 3 days of tone-shock extinction (resembling ERP). On the 4th day, the platform barrier is removed to test for success of Ext-RP (no avoidance) or failure of Ext-RP (persistent avoidance). Of 60 rats that underwent Ext-RP, 15 (25%) showed persistent avoidance at test. These persistent rats also showed higher freezing to the tone during Ext-RP. Persistent avoidance could be eliminated by returning the barrier to the test chamber (away from the platform), suggesting that the barrier acts as a safety signal. DBS of the ventral capsule/ventral striatum (VC/VS) has been shown to improve response to ERP in patients that previously failed ERP (Greenberg et al., 2006; Denys et al., 2010). We therefore applied DBS to VS in persistent avoidance rats, during an additional Ext-RP session, in an attempt to revert persistent avoidance. DBS did not reduce freezing during Ext-RP, but abolished persistent avoidance, as compared to sham controls (0% vs 66% time avoiding, $p<0.01$). Excessive fear and dependence on safety signaling resemble OCD patients, suggesting that this model may be useful to characterize the neural mechanisms of persistent avoidance of triggers present in pathological anxiety.

Disclosures: **J. Rodriguez-Romaguera:** None. **H. Bravo-Rivera:** None. **E.I. González-Araya:** None. **A.M. Minier-Toribo:** None. **B.D. Greenberg:** None. **S.A. Rasmussen:** None. **G.J. Quirk:** None.

Poster

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Title: Neural correlates of conditioned fear retrieval in the paraventricular thalamus

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Abstract: Pharmacological inactivation of the dorsal midline thalamus (dMT) impaired fear retrieval when performed 24 h after fear conditioning, but not 2 h after (Padilla-Coreano et al 2012). These results suggest that one or more structures within dMT are recruited into the fear circuit after conditioning. Consistent with this, the paraventricular nucleus of the thalamus (PVT), a subregion of dMT, showed increased expression of the neural activity marker cFos 24 h after conditioning, but not 6 h after (Do Monte et al, SfN poster, 2013). cFos measurements are limited because they cannot track the same neurons at different timepoints. We therefore used single unit recording to track PVT neurons before, 2 h after, and 24 h after fear conditioning. Regarding spontaneous firing rate, more neurons showed increases 24 h after conditioning (51%), compared to 2 h after (16%; Fisher's exact, $p= 0.034$, $n= 37$), consistent with cFos findings. In contrast, the percentage of cells showing conditioned tone responses ($Z > 2.58$ in the first two sec after tone onset) was the same at both 2 h and 24 h timepoints (16%). Interestingly, the neurons that were tone-responsive at 24 h were not tone-responsive at 2 h, and vice versa, suggesting that distinct PVT ensembles may be recruited over time. Thus, time-dependent changes in both spontaneous and tone-induced firing are consistent with time-dependent recruitment of PVT neurons for retrieval of conditioned fear.

Disclosures: **K. Quinones-Laracuente:** None. **F.H. Do Monte:** None. **G.J. Quirk:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Differential rearing affects pCREB expression in the rat nucleus accumbens following fear extinction

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Abstract: Early life experiences can affect the development of anxiety disorders in predisposed individuals. Rats that are isolated during the rearing period are prone to fearful behavior, suggesting that the ability to regulate fear can be mediated by the rearing environment (Fone & Porkess, 2008). Differential rearing also influences the expression of phosphorylated cAMP response-element binding protein (pCREB), which is a transcription factor involved with learning and memory. Although enrichment typically increases pCREB expression, previous literature has reported that isolated rats have greater expression of pCREB within the nucleus accumbens (NAcc) than enriched rats (Green et al., 2010). The present study determined whether enriched and isolated rats express pCREB differently within the NAcc following fear extinction. We hypothesized that enriched rats would extinguish fear at a faster rate due to enhanced learning, but that isolated rats would express more pCREB within the NAcc following fear extinction. Male Sprague-Dawley rats at 21 days of age were assigned to an enriched condition (EC) or an isolated condition (IC). EC rats were housed together with novel objects and handled daily while IC rats were housed individually and not handled throughout the rearing period. At 51 days of age, rats were trained to lever-press for 20% sucrose on a variable-interval schedule of reinforcement. Across 2 fear acquisition sessions, rats received 8 presentations of 3000 kHz tone (CS) paired with 0.6 mA foot shock (US). Rats then received 8 presentations of tone only during 1 fear extinction session. An unpaired CS/US control group of rats was included to investigate pCREB expression in the NAcc in the absence of fear. Rats in the unpaired CS/US control group received the same number of CS and US presentations during acquisition and extinction sessions, but did not learn that the tone predicted the shock. We predicted that there would be no differences in pCREB expression between EC and IC rats in the unpaired control group. Immediately following all fear extinction sessions, brains were extracted and the expression of pCREB was quantified within the NAcc. Results revealed no differences in fear extinction rate between EC and IC rats; however, IC rats expressed more pCREB in the NAcc

compared with EC rats following the extinction of fear. There were no differences in pCREB expression in the unpaired group between EC and IC rats in the absence of fear. These findings suggest that differential rearing environments may be uniquely affecting pCREB expression in the NAcc following fear extinction.

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Poster

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Pritzker Neuropsychiatric Disorders Research Consortium

Hope for Depression Research Foundation

Title: Fibroblast growth factor 2 enhances the retention of extinction learning in resilient but not vulnerable rats bred for their locomotor response to novelty

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Abstract: As we search for new treatments for anxiety disorders, it is important that we understand why certain individuals are more vulnerable to developing PTSD after a traumatic event. We believe that our rat model of individual differences in locomotor response to a novel environment may provide insight into vulnerability and resilience to anxiety disorders. In experiment one, rats selectively bred for high (bHR) or low (bLR) locomotor response to a novel environment and rats from the F1 generation of a bHR-bLR cross (bIRs), which display an intermediate locomotor phenotype, received a standard fear conditioning and extinction paradigm. bLRs and bIRs demonstrated significantly higher freezing than bHRs during

conditioning, while bHRs exhibited a more active coping strategy, having higher levels of escape behavior during the conditioning session. bLRs demonstrated decreased extinction learning and extinction retention than bIRs and bHRs, while bHRs exhibited faster extinction and greater retention than bIRs. This indicates that bLRs may be vulnerable to maladaptive fear behaviors, while bHRs may be more resilient. Fibroblast growth factor 2 (FGF2) facilitates extinction learning in outbred rats when given acutely, and reduces spontaneous anxiety behavior in these selectively bred animals when given in early life. We hypothesized that FGF2 might normalize bLR extinction behavior. In experiment two, bHRs and bLRs were given FGF2 (20 ng/g) or vehicle (0.1M PBS) subcutaneously on the day after birth, then given a standard fear conditioning paradigm in adulthood. Phenotypic differences in fear and extinction behavior remained strong, with bLRs showing reduced extinction learning and retention compared to bHRs. Perinatal FGF2 administration facilitated extinction retention in bHRs, but not in bLRs. FGF2 administration modified unlearned, spontaneous, anxiety-like behavior in the bLRs. These data suggest that genetic background is an important determinant of FGF2 modulation of anxiety and fear behavior. Future studies are investigating the gene and protein expression profiles of these animals to understand the brain mechanisms behind their differential behavior. The individual differences in fear and extinction learning seen in these rats provide an important model for developing understanding of underlying differences in vulnerability to maladaptive fear and the role of the fibroblast growth factor family in modulating fear conditioning and extinction behavior.

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Poster

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Environmental enrichment ameliorate numbing behavior on an animal model of posttraumatic stress disorder using a shuttle box in rats

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Abstract: OBJECTIVES: Using a shuttle box, rats exposed to inescapable footshocks (IS) corresponding to trauma 2weeks before had the persistent behavioral alterations characterized by 'bi-directional changes', especially in relation to 'numbing' and 'hyperarousal' symptoms of posttraumatic stress disorder (PTSD). Due to these findings, these rats could serve as a useful model of PTSD. There are several reports that Environmental enrichment (EE) ameliorates the PTSD symptoms although it has not yet been reported that how EE effects the 'bi-directional' behavior of PTSD. In this study, we examined the preventive effects of EE on PTSD-like behaviors in our PTSD model of rat. METHODS: Two weeks after the IS session corresponding to trauma, the avoidance/escape task trials were performed. Detailed experimental procedures were described as previously (Sawamura et al, 2004). After the IS session, EE groups were housed in large cage (40 x 54 x 28 cm) with groups of 6, containing 9 wooden and plastic objects for two weeks. Elevated plus maze test and locomotors activity test was also performed after the period of 2weeks EE. RESULTS: In the elevated plus maze test, a significant increase in the % of time spent in the open arm and in the locomotors activity test, a significant decrease in the total distance moved in the field was observed in EE groups ($p < 0.05$). Two weeks of EE treatment significantly ameliorated numbing-like behaviors ($p < 0.05$) although it did not recover from hyperarousal-like behaviors. Moreover, EE treatment significantly changed 'bi-directional changes' in our PTSD model. CONCLUSIONS: These findings suggested that EE might be an effective treatment for PTSD, especially for numbing-like symptoms. REFERENCES:
Sawamura et al. (2004) Effect of paroxetine on a model of posttraumatic stress disorder in rats. Neuroscience Letters, 357: 37-40.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

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Title: Pharmacogenetic excitation of basolateral amygdala glutamatergic neurons mimics positive prediction error to enable fear learning

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Abstract: Prediction error, or the discrepancy between expected and actual outcomes of a conditioning trial, is integral in causing fear learning. It is widely assumed that during fear learning such prediction error drives variations in the activity of BLA glutamatergic neurons responsible for CS - US association formation. Here we combined pharmacogenetic manipulation of BLA glutamatergic neurons with a behavioural associative blocking design to determine the causal role of BLA glutamatergic neurons in fear prediction errors. Blocking involved training rats to fear conditioned stimulus (CS) A in Stage I via pairings with shock. In Stage II, rats received pairings of CSA+CSB and shock. Blocking was shown by less fear to CSB at test relative to a control group that received Stage II, but not Stage I, training. Rats received BLA application of either AAV5-CamKII-hM3Dq-eYFP or AAV5-CamKII-eYFP prior to the commencement of a behavioural blocking procedure. Immediately prior to Stage II rats received i.p. injections of clozapine-N-oxide (3mg/kg; CNO) or vehicle. AAV5-CamKII-eYFP rats showed blocking of fear learning to CSB regardless of whether they were treated with CNO or vehicle prior to Stage II. In contrast, AAV5-CamKII-hM3Dq-eYFP rats showed attenuation of blocking at test when treated with CNO during Stage II, but not with vehicle. Thus, pharmacogenetic excitation of BLA glutamatergic neurons prevented blocking of fear learning. To determine whether this was due to an AAV5-CamKII-hM3Dq-eYFP change in processing the shock US, asymptotic levels of fear learning, or fear memory consolidation, we studied the effects of pharmacogenetic excitation of BLA glutamatergic neurons on the acquisition of simple fear conditioning. Two groups of rats expressing either AAV5-CamKII-hM3Dq-eYFP or AAV5-CamKII-eYFP were treated with CNO (3mg/kg) prior to CS - shock pairings. These rats did not differ in either the rate of acquisition or asymptotic levels of fear. These results show that manipulations that increase activity of BLA glutamatergic neurons mimic positive prediction error to enable fear learning.

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Poster

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MEXT SRPBS

RIKEN BSI

Title: An amygdala-periaqueductal gray circuit for calculating prediction errors in amygdala neurons and setting the strength of fear memories

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Abstract: Elucidating how adaptive levels of fear memory strength are set is vital for our understanding of anxiety disorders which are typified by exaggerated aversively motivated learning. During auditory fear conditioning, animals learn that an auditory tone predicts an aversive outcome (electric shock). Learning reaches a steady state at a certain memory strength (termed the learning asymptote) beyond which further training is ineffective at producing learning unless the strength of the aversive outcome is increased. We previously found a learning dependent reduction in shock processing (prediction error coding) in lateral amygdala (LA) neurons. Because activation of LA neurons by aversive outcomes is thought to trigger plasticity at auditory input synapses to the LA, it is possible that this reduction sets learning asymptotes. However, it is not clear what neural circuit mechanisms compute prediction errors and if this sets learning asymptotes. Similar prediction error coding also occurs in the periaqueductal gray (PAG) which relays aversive shock signals to LA. PAG receives inhibitory input from central nucleus of amygdala (CeA) which is activated by tones after fear learning. Here, we hypothesized that a CeA-PAG pathway provides a negative feedback on PAG to produce prediction error coding in LA neurons and that this neural coding in LA sets fear learning asymptotes. We first developed a 4-day fear conditioning paradigm in which rats were trained (days 1, 3) and tested (days 2, 4) twice. In this paradigm, rats reached learning asymptote after the initial training (day 1) as learning was not enhanced by overtraining (day 3). Further, we confirmed that even at learning asymptote larger-than-predicted shocks induced stronger activation of LA neurons (i.e. reengaged prediction error coding) and increased learning asymptotes. Using this behavioral paradigm, we found that: (1) optogenetic inhibition of CeA-PAG afferents at learning asymptote increased learning levels and that this manipulation also

reengaged prediction error coding in LA neurons. (2) optogenetic additive activation of shock-evoked responses in LA pyramidal cells during overtraining also increased learning asymptotes. (3) optogenetic inhibition of shock-evoked LA neuronal activity during overtraining abolished the increase in fear learning induced by a higher shock intensity. These results support the hypothesis that a CeA-PAG negative feedback pathway regulates prediction error coding in the LA to set learning asymptotes. This suggests a circuit mechanism for setting adaptive levels of fear memory strength and shows that disrupting this circuit produces exaggerated levels of fear.

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Poster

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Title: *In vitro* conditioned inhibition changes in Hermissenda type b photoreceptors

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Abstract: Little is known about the mechanisms underlying conditioned inhibition (CI), where an organism learns that one stimulus signals the absence of a second.. In aversive Pavlovian conditioning, CI learning is often referred to as safety-signal learning. Previous research has shown that CI in *Hermissenda (H.c.)*, established by repeated explicitly-unpaired (EU) presentations of light (CS) and rotation (US), separated by a fixed, lengthy temporal gap, results in increased phototactic behavior and decreased photoresponses and spike activity of ocular Type B photoreceptors (Britton & Farley, 1999, *J Neurosci*; Walker et al., 2010, *Front Behav Neurosci* .) Recent studies (Farley et al., 2014) have determined the ionic conductance changes that underlie the maintained decreases in B cell light-evoked generator potentials (SSGPs) and spike frequencies due to EU training: increases in two somatic K^+ currents (I_A and $I_{K\text{-}Ca}$). Increases in I_A were sufficient to account for decreased spiking, while increases in both I_A and $I_{K\text{-}Ca}$ contributed to the decreased SSGP. Additional studies implicated increased activities of PP1, PP2B, and arachidonic acid/12-lipoxygenase metabolites (AA/!2-LOX) as crucial for increased

I_A and reductions in spiking. Here, I report on the changes observed in Type B cells during *in vitro* EU-conditioning of the isolated CNS. EU conditioning was simulated by exposing a CNS to ten EU-presentations of light (30 sec ; ~ 300 uW/ cm^2) and intracellular current stimulation (30 sec) of a statocyst caudal hair cell, sufficient to produce high frequency trains of action potentials. The ISI between light and hair cell stimulation was 5.0 min, and the ITI was 10 min. Control preparation were exposed to ten Random presentations of the same light and hair cell stimuli, at the same overall frequency. B cells (n=7) from EU-conditioned CNSs showed an average: 7-8 mV hyperpolarization of resting V_m, a 5 mV decrease in SSGP, and a 32% decrease in spike frequency. In contrast, Random (n=3) CNSs showed a 2 mV depolarization of V_m, a 2 mV increase in SSGP, and a 5% increase in spiking. The EU-produced alterations in excitability and SSGP were abolished when either EGTA or a calmodulin inhibitor was included in the recording electrode, implicating Ca^{2+}-dependent signaling pathways as essential. Related ratiometric Ca^{2+}-imaging studies of B cells implicated a time-dependent decrease in intracellular Ca^{2+} (4-6 min post light offset) as a crucial permissive signal for EU-produced decreases in B cell excitability, if coincident with hair cell stimulation. Several signaling models of "non-coincidence" and CI learning are currently being tested in this *in vitro* preparation.

Disclosures: J. Farley: None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

Title: Neuronal morphology and GSK-3b signaling associated transcripts are differential features in the hippocampus of fear-resilient mice during fear conditioning

Authors: S. L. DASH[1], D. JACOBOWITZ[1], R. URSANO[2], *C. L. DALGARD[3]
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Abstract: Post traumatic stress disorder (PTSD) affects approximately 8.6% of veterans returning from conflicts in Iraq and Afghanistan. Intrusive fearful thoughts and memories are one of the hallmarks of PTSD. Accordingly, animal models of fear memory are utilized to investigate this component of PTSD in controlled environments. To study the mechanisms of fear conditioning, which mimics certain symptoms of PTSD, an advanced intercross mouse F8 line of

C57BL/6J and DBA/2J was generated to select for fear susceptibility or resilience by selective breeding . These fear susceptible and fear resilient mice were previously utilized to identify candidate genes that contribute to baseline susceptibility or resilience to fear establishment. Functional imaging in these mice demonstrated differential activity in the hippocampus of the fear susceptible mice, which amygdala activity was non-significantly different. To investigate the mechanisms underlying this difference in activity we performed complete transcriptome profiling using RNA-seq of hippocampal tissue from these mice and performed differential expression analysis to identify candidate transcripts that may be associated with the functional activity differences observed. An enrichment of transcripts associated with neuronal morphology, GSK-3b associated cellular signaling and axonal transport was detected. Comparative analysis of transcriptomes as a function of fear conditioning exposure revealed a distinct subset of transcript features enriched for STAT associated intracellular signaling. We anticipate that functional analysis of these candidate transcripts for fear phenotypes and identification of systems biology level traits will elucidate novel strategies to mitigate deleterious mechanisms contributing to developing PTSD.

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Poster

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Support: R00 AG039511

Title: Upregulation of Kv12.2 following contextual fear conditioning and implications for Alzheimer's Disease memory decline

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Abstract: More than 5 million elderly people in the United States are currently affected by Alzheimer’s disease (AD), and by 2050 this number is expected to rise to ~16 million (Alzheimer’s Association, 2012). Aging is the most important risk factor for AD dementia, and age-associated changes in protein expression play a role in this memory impairment. The

working hypothesis in our lab suggests that the memory deficits that underlie AD and 'normal' aging may share a common mechanism. There is a discrepancy regarding the role of the voltage-gated potassium channel, Kv12.2, in spatial memory based on genetic manipulations. To this end, we utilized un-biased discovery proteomics (the large-scale study of proteins), contextual fear conditioning, and western blot analyses to test the hypothesis that fear conditioning would alter Kv12.2 hippocampal protein expression. Data from the mapped hippocampus membrane proteome of AD strong and weak-learners (n = 9 mice per group) found that the Kv12.2 was significantly differentially expressed relative to memory performance, and a subsequent analysis revealed a 4.8-fold increase in Kv12.2 in AD weak-learners vs. strong-learners ($p = 8.5 \times 10^{-10}$). Similarly, there was a negative relationship between Kv12.2 expression and performance on both the hippocampal-dependent water maze (n=10 strains) and fear conditioning (n=20 strains) tasks in adult and aged BXD mice (C57BL/6J x DBA/2J recombinant inbred strains), respectively. Interestingly, however, adult male C57BL/6J mice that exhibited enhanced freezing with fear conditioning, indicating enhanced fear memory, showed a significant increase in hippocampal Kv12.2 protein expression ($p<0.05$). Collectively, our results suggest that that there may be a disease related change in Kv12.2 expression. Further tests will be conducted to elucidate the mechanism by which Kv12.2 affects memory.

Disclosures: L.A. Wilmott: None. **S.M. Neuner:** None. **K.A. Hope:** None. **C.C. Kaczorowski:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 467.21/UU80

Topic: F.02. Animal Cognition and Behavior

Support: INDICASAT-doctoral fellowship IFARHU-SENACYT

Title: Learned fear and safety in rats: Role of neurotransmitters and BDNF expression

Authors: *C. E. VASQUEZ[1,2], R. RIENER[3], J. RICCA[4], R. COSSIO[1], G. B. BRITTON[1]
[1]INDICASAT AIP, Panama, Panama; [2]Acharya Nagarjuna Univ., Guntur, India; [3]Univ. of Santa Cruz, Santa Cruz, CA; [4]Lafayette Col., Easton, PA

Abstract: Learned safety has been shown to produce anti-depressant effects in mice, and the neurocircuitry involved has not been fully explored. The objective of the present study was to

examine the effects of fear and safety learning process in the levels of neurotransmitters and brain derived neurotrophic factor (BDNF) expression. Young adult male rats were trained in fear (FC) and safety conditioning (SC) paradigms. FC animals were trained with tone and footshock pairings. SC animals received explicitly unpaired tone and shock presentations. Fear memory (freezing) was measured during pre-tone, tone and post-tone periods in the training context in the absence of shock. Our results showed a reduction of freezing during the tone in the SC group and elevated levels of fear to the tone in the FC group. Retardation tests confirmed that the safety signal produced fear inhibition. In addition, 4 hours after training we measured levels of norepinephrine (NE) and 5-hydroxyindoleacetic acid (5 HIAA) in prefrontal cortex (PFC) and hippocampus (Hip), part of major neuroanatomical pathways of anxiety and depression. 5 HIAA levels were similar across groups and areas. However, FC reduced NE levels in the Hip. In SC, we observed a tendency toward reduced NE levels that did not reach significance. We also determined the effects of FC and SC on the expression of BDNF mRNA at 4 h after training; both FC and SC increased BDNF mRNA in the PFC to approximately 4 and 5 fold respectively. In the Hip only FC increased BDNF mRNA levels, suggesting a role of hippocampal BDNF in FC but not SC. Together these results indicate SC selective induction of PFC BDNF mRNA as a one of the mediating factors during safety learning. In contrast, learned fear-induced increases in both PFC and Hip BDNF mRNA suggest dual contribution of PFC and Hip BDNF mRNA during fear memory consolidation. Ongoing studies are focused on BDNF expression changes associated with FC and SC learning after 24 h of training and the expression of BDNF receptor tropomyosin-related kinase B at 4h and 24 h.

Disclosures: C.E. Vasquez: None. **R. Cossio:** None. **G.B. Britton:** None. **J. Ricca:** None. **R. Riener:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Topic: E.05. Stress and the Brain

Support: Farris Family Award

Whitehall Foundation Grant

Title: Neuroendocrine and gene expression changes associated with delayed susceptibility and sustained resilience to juvenile social defeat

Authors: *M. S. LATSKO, S. HAYNIE, L. FARBAUCH, A. JASNOW
Kent State Univ., Kent, OH

Abstract: Anxiety is important for health and survival, but can become excessive, as exemplified by Post Traumatic Stress Disorder (PTSD). Anxiety disorders can present early in life, and can influence judgment and interfere with social relationships. Moreover, there is a very strong relationship between the level of childhood trauma and adult anxiety disorder symptoms in humans. In the current study, we used acute social defeat as an animal model of trauma and stress to investigate the effects of social stress on subsequent social behavior in adolescent and adult mice. Male C57Bl6/J mice, (PND 28±2) were subjected to social defeat and tested 24 hours later for social interaction and tested again 30 days later as adults. Socially stressed juveniles initially exhibit normal levels of social interaction, suggesting immediate "resilience," to the effects of social defeat. However, when these mice are tested again as adults, a delayed susceptible (low social interaction) phenotype develops in most of the population. An additional phenotype develops, one in which the mice are stably resilient to the effects of social defeat when tested as juveniles and as adults. We are currently examining whether these phenotypes are associated with differential levels of neuroendocrine markers, such as corticosterone and testosterone, or patterns of gene expression and epigenetic alterations. These experiments will identify distinct physiological indicators of these two phenotypes, improving understanding of how stressful early life experiences interact with hormonal and neuroepigenetic components to influence ontogeny of maladaptive behavioral outcomes.

Disclosures: M.S. Latsko: None. **S. Haynie:** None. **L. Farbauch:** None. **A. Jasnow:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Program#/Poster#: 467.23/UU82

Topic: F.02. Animal Cognition and Behavior

Support: RFBR Grant 13-04-01892A

RFBR Grant 13-04-40334-H

Title: Behavioral investigation of complex associative memory in tone-light compound fear conditioning in mice

Authors: *K. TOROPOVA[1], O. IVASHKINA[1], M. ROSHCHINA[1], K. ANOKHIN[1,2,3,4]
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Abstract: Much of research on molecular and cellular mechanisms of associative memories involves Pavlovian conditioning to a single, simple conditioned stimulus; yet, for the animal behaving in natural environment, most associative memories are complex_they integrate elements from a number of sensory modalities. Here we developed a model for multisensory compound conditioning in mice that mimics features of natural associative learning, and evaluated properties of resulting complex memories. For this purpose, we used a cued fear conditioning paradigm with a compound conditioned stimulus (CS) that consisted of auditory (tone, T) and visual (blinking light, L) components. First, we showed that mice could establish fear memories to compound tone-light (T+L) stimulus under conditions of various tone, light and foot-shock intensities, whereas learning to the light CS was slower than to the sound CS. Next, we studied differential freezing response of mice to the compound CS and its components 1 day or 7 days after training. Finally, we tested whether between-stimuli association could be attenuated through extinction of tone, light, or T+L in our compound fear conditioning model. The amount of freezing to individual components (T or L) after the compound (T+L) conditioning differed significantly from freezing to the same stimuli after mice were trained to them separately (T CS or L CS). Thus, mice were able to differentiate between stimuli used as discrete conditioning cues and the same stimuli used as components of the compound conditioning stimulus. After conditioning to the compound stimulus (T+L) freezing to the light component was weaker than to the tone component. Additionally, one day after training mice did not have a pronounced response to the light component and had decreased freezing response to the sound component. These responses to the components of the compound developed only a week later. Thus, memory of compound CS has “matured” over a week after training. Seven days after fear conditioning to the compound tone-light cue (T+L), mice had distinctive freezing response to the compound stimulus and its individual isolated components (tone, light, and T+L). Animals remembered separately both components of the compound CS and testing to the one component (T or L, but not T+L) did not extinguish the memory for the second.

Disclosures: K. Toropova: None. **O. Ivashkina:** None. **M. Roshchina:** None. **K. Anokhin:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Program#/Poster#: 467.24/UU83

Topic: F.02. Animal Cognition and Behavior

Support: NIEHS grant ES04184

GVSU grant-in-aid

Title: Embryonic lead exposures cause learning deficits in adult male and female zebrafish

Authors: N. MERCADO-IDZIAK[1], J. BLUCHER[1], D. WEBER[2], *X. XU[1]
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Abstract: The zebrafish has become a useful organism for studying the effects of environmental contaminants on the neurobehavioral development of an organism due to its short generation times, high numbers of eggs per female, ease of breeding, and short developmental periods before hatching. The present study investigated the effects on learning due to embryonic exposure to lead (Pb^{2+}) in adult male and female zebrafish using avoidance conditioning as the behavioral paradigm. Adult zebrafish were trained to associate light with shocks in a fish shuttle-box consisting of a water-filled tank separated by a barrier into two equal compartments. A trial began with the onset of light on the side of the fish's location and the manually raised barrier; 12 seconds later repetitive electrical shocks were administered. Fish initially swam through the barrier after receiving several shocks. After repeated trials, fish learned to swim from the lighted end to the dark end before the administration of shocks to avoid the body shock, which is called avoidance response. Two days later, fish were tested for avoidance responses. In Experiment 1, adult male zebrafish that were exposed to 0, 0.1, 1, or 10 μM Pb^{2+} as embryos (2-24 hours post fertilization) were trained and tested for avoidance responses. The results showed that male zebrafish hatched from embryos exposed to no lead learned avoidance responses during training and showed significantly increased avoidance responses during testing. Male zebrafish hatched from embryos exposed to Pb^{2+} showed no significant increases in avoidance responses from training to testing. In Experiment 2, adult female zebrafish that were exposed to an identical exposure regimen as in Experiment 1 were trained and tested for avoidance responses. The results showed that female zebrafish hatched from embryos exposed to no lead learned avoidance responses during training and showed increased avoidance responses during testing, while female zebrafish hatched from embryos exposed to Pb^{2+} showed no significant changes in avoidance responses from training to testing. The pooled results of both experiments showed that embryonic Pb^{2+} exposure produced learning impairments in a concentration-dependent manner.

Disclosures: **N. Mercado-Idziak:** None. **J. Blucher:** None. **X. Xu:** None. **D. Weber:** None.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

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Program#/Poster#: 467.25/UU84

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Title: Calcium imaging of stimulus specific neuronal responses in the lateral amygdala during fear learning

Authors: *B. F. GREWE[1], J. LECOQ[1], L. KITCH[1], J. LI[1], J. MARSHALL[1], G. VENKATARAMAN[1], J. GRÜNDEMANN[2], A. LÜTHI[2], M. J. SCHNITZER[1]
[1]Stanford Univ., Palo Alto, CA; [2]FMI, Basel, Switzerland

Abstract: During fear learning and extinction the lateral amygdala (LA) has a crucial role as a primary site of neural plasticity. However, *in vivo* electrophysiological recordings probing learning-related neural dynamics have typically been limited to handfuls of individual neurons in LA that were tracked over modest time periods (e.g. 1-3 days). Here we imaged the concurrent dynamics of substantial populations of individual LA neurons by using a miniature (2 gram) fluorescence microscope [Ghosh et al. Nature Methods (2011) 8:871-8] in combination with a chronic mouse preparation for time-lapse fluorescence microendoscopy [Ziv Y et al., Nature Neuroscience (2013) 16:264-6]. By targeting the expression of the genetically encoded calcium indicator GCaMP5 to LA excitatory neurons, we were able to monitor up to ~180 individual neurons per mouse across multiple days and weeks. Our extended experimental protocol for combined fear conditioning and calcium-imaging included: two habituation and baseline imaging sessions; a session of classical cued fear conditioning in which mice received paired presentations of an auditory tone and electrical foot shock; and multiple testing sessions that probed fear memory retrieval and extinction, as scored by the mouse's freezing responses. The stability of the microscope on the cranium was sufficient to monitor neural dynamics even when the mouse reacted vigorously to the electric shock, enabling us to extract neural responses to the shock as well as to the tone. Two distinct, but overlapping neural populations had evoked responses to the two stimuli, and we are presently examining their potential contributions to the neural representation of fear learning. Overall, our methodology permits repeated, long-term imaging of neural populations deep in the brain, permitting unprecedented analyses of large-scale neural coding in genetically specified cell types. In the amygdala, our approach opens the door to time-lapse imaging studies of neural coding in a brain structure that likely plays a central role in several psychiatric disorders.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 467.26/UU85

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant MH62044

Title: Is conditioned defeat associated with differential activation of brain regions that influence resilience or susceptibility to stress?

Authors: ***B. M. THOMPSON**, K. E. MCCANN, K. L. HUHMAN
Neurosci. Institue, Univ. of Georgia, Atlanta, GA

Abstract: Social defeat in Syrian hamsters is an ethologically relevant model for studying behavioral and brain responses to acute stress. Hamsters readily display territorial and agonistic behavior towards a conspecific, but a brief defeat experience will produce a salient change in their behavior such that a previously defeated hamster will subsequently display marked submission even to a smaller, non-aggressive intruder. This shift in behavior has been termed conditioned defeat (CD). In both humans and animals, however, not every individual who experiences stress or trauma suffers deleterious effects; similarly, hamsters also display a range in the expression of CD. The ability to withstand stressful events without developing long-term behavioral or neurophysiological changes is known as resilience. This study focused on the neural substrates that underlie resilience to the development of CD. Animals were exposed to a 15-min defeat. One hour later, they received a 5-min exposure to a non-aggressive intruder and submissive behavior was recorded (CD). Controls received neither the defeat session nor CD test. Animals were sacrificed immediately following the completion of the test for CD and brains were collected for immunohistological analysis of the immediate early gene product c-Fos. The timing of this experimental design allowed us to identify brain regions activated during the defeat experience that may account for variations in the expression of CD. Although a few studies have examined differences in c-Fos in various brain regions in dominant and submissive

animals following an agonistic encounter, we were particularly interested in neural activity that corresponds to the resilience to the development of CD (i.e., animals that show high vs. low levels of CD despite similar previous defeat). Thus, animals were divided into groups of High or Low CD based on their behavior. We analyzed c-Fos expression in various neural substrates that are thought to underlie resiliency, such as the infralimbic and prelimbic medial prefrontal cortex, as well as in several stress-responsive regions known to be important in the acquisition of conditioned defeat, such as the basolateral amygdala. We present differences in region-specific activation associated with high or low levels of CD. The present results contribute to an understanding of the neural substrates that underlie resilience, which is critical for the treatment and prevention of stress-related psychological disorders.

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Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 22500378

KAKENHI 23300249

Title: Tickling alters fear-related and cognitive behaviors in rats isolated during adolescence

Authors: ***M. HORI**[1], K. YAMADA[2], J. OHNISHI[3], H. FURUIE[2], K. MURAKAMI[1], Y. ICHITANI[2]
[1]Fndn. For Advancement of Intl. Sci., Tsukuba / Ibaraki, Japan; [2]Inst. of Psychology and Behavioral Neurosci., Univ. of Tsukuba, Tsukuba, Japan; [3]Dept. of Food and Nutr., Tokyo Kasei Univ., Tokyo, Japan

Abstract: Social interactions are important for neuronal development and behavior. Especially, play behavior during juvenile and adolescence is considered to facilitate neural and social development, whereas social isolation is noxious and increase the stress vulnerability in rodents. Previously, we showed that positive emotions induced by tickling which resembles the rat's play could modulate fear-related behaviors and sympatho-adrenal stress responses. In the present study, we focused on how tickling during adolescence affects stress vulnerability induced by

socially isolated rearing. First of all, we investigated whether 4 weeks of tickling alters conditioned fear responses. The group-housed and isolated-tickled rats showed a significant decrease in freezing response in the 96-h retention test compared to the 48-h retention test performed after conditioning; however, isolated-nontickled rats did not. The current study demonstrated again that tickling might facilitate fear extinction. To examine the effects of tickling on hormonal responses associated with conditioned fear, we measured plasma catecholamine and corticosterone levels after conditioning and the 96-h retention test. We found that tickling stimulation inhibited stress-induced increment of adrenaline and noradrenaline levels immediately after conditioning. Conversely, no differences in plasma corticosterone levels were observed among groups. Next, in order to examine whether tickling has any effects on memory function, we tested the Morris water maze learning at two stages of their development, late adolescence and adulthood, since isolation stress influences spatial cognition and memory and alters hippocampal development and neurogenesis. No differences among groups were found in the escape latency of training period, the retention test and its reversal learning when rats were trained in late adolescence. On the other hand, if rats were trained in adulthood, mean escape latency on day 3 was longer in isolated animals, but not in tickled animals, compared with group-housed animals. These results demonstrated that socially isolated rearing altered the performance in Morris water maze in adulthood, and tickling treatment improved the deficits. Taken together, it is suggested that tickling treatment during adolescence is able to reverse/protect increase of stress vulnerability that is caused by isolated rearing.

Disclosures: **M. Hori:** A. Employment/Salary (full or part-time):; Foundation for Advancement of International Science. **J. Ohnishi:** A. Employment/Salary (full or part-time):; Tokyo Kasei University. **K. Yamada:** A. Employment/Salary (full or part-time):; University of Tsukuba. **Y. Ichitani:** A. Employment/Salary (full or part-time):; University of Tsukuba. **H. Furuie:** None. **K. Murakami:** A. Employment/Salary (full or part-time):; Foundation For Advancement of International Science.

Poster

467. Fear and Aversive Memories: Acquisition and Extinction

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Topic: F.02. Animal Cognition and Behavior

Support: ICMR Grant- 45/05/2010/PHY-BMS

Title: Stress during stress hyporesponsive period (SHRP) attenuates both retention and extinction of fear memory in rats

Authors: *P. K. MISHRA, JR, B. M. KUTTY, L. T. RAO
NIMHANS, Bangalore, India

Abstract: Purpose: The long-term impact of 10-days of maternal separation stress on the fear memory and fear extinction was evaluated in male Wistar rats. **Methods:** Early maternal separation and isolation stress (EMS) were carried out in rat pups during the Stress Hypo Responsive Period (SHRP) for 6 hours daily for 10 days, while effects on fear retention and extinction were ensured 2 months later. 2 months after EMS, rats were exposed to cued fear conditioning session. 24 hours, 48 hours and 72 hours after fear conditioning, both normal control and EMS groups of rats were received extinction training sessions respectively. Percentage freezing was assessed during all stages of fear retention and fear extinction training including session of retention of fear extinction. **Results:** The retention of fear memory was stronger in EMS rats than controls. 10 days after extinction training session, EMS rats showed increased freezing to the conditioned stimulus than controls. **Conclusions:** Our data suggested that EMS causes a long-term behavioural disposition that is activated by acute stressors like fear conditioning.

Disclosures: P.K. Mishra: None. **B.M. Kutty:** None. **L.T. Rao:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant P50MH079513

BBRF Grant NARSAD Young Investigator Award

NIMH Grant K99 MH097822

Title: Sensitive period for fear regulation during adolescence

Authors: *S. S. PATTWELL[1], C. LISTON[2], D. JING[2], I. NINAN[3], B. J. CASEY[2], K. DEISSEROTH[4], F. S. LEE[2]

[1]Human Biol., Fred Hutchinson Cancer Res. Ctr., Seattle, WA; [2]Weill Cornell Med. Col., New York, NY; [3]New York Univ. Sch. of Med., New York, NY; [4]Stanford Univ., Stanford, CA

Abstract: The only evidence-based behavioral treatment for anxiety and stress-related disorders relies on desensitization techniques based on principles of extinction learning, yet as many as 40% of patients do not respond to this treatment. Converging evidence from human and rodent studies suggests that insufficient top-down regulation of subcortical structures, such as the amygdala, coincides with diminished prototypical fear extinction learning. Because this top-down prefrontal regulation mediates successful extinction learning and may determine the efficacy of exposure therapy, often used as part of cognitive behavioral therapy (CBT), it is important to discern how immaturity in this regulatory circuitry in developing populations influences fear extinction. Efforts have focused on individual differences in treatment response, but not on when during development treatments may be most effective. Parallel behavioral studies have revealed attenuated cue extinction learning during adolescence, in both humans and mice, compared to younger and older age groups, while developmental changes within the neural circuitry implicated in fear regulation reveals diverging responses to contextual and cue elements. Through probing developmental changes in structural connectivity within the fear neural circuitry via retrograde tract tracing and utilizing *in vivo* imaging techniques to explore dendritic spine dynamics in developing mice, we hope to provide insight toward optimizing treatment outcomes according to when, during development, specific types of exposure therapies may be most effective.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Title: Acute effect of X-irradiation and carbon ion-irradiation on fear memory formation and its underlying mechanism

Authors: A. PUSPITASARI[1], N. KOGANEZAWA[1], N. KOJIMA[1,3], M. ISONO[2], Y. YUKARI[2], *T. SHIRAO[1]
[1]Dept Neurobio. & Behavior, Gunma Univ. Grad Sch. Med., Maebashi, Gunma, Japan; [2]Heavy

Ion Med. Ctr., Gunma Univ. Grad Sch. Med., Maebashi, Japan; [3]Dept. of Life Sci., Toyo Univ., Itakura, Japan

Abstract: Heavy ion therapy is becoming widely known and used as the advanced therapy for cancer. However, the effects of heavy ion beam on healthy neurons surrounding the cancer and on higher brain function are still poorly understood. To compare the effects of X-irradiation and heavy ion beam on the brain, we conducted *in vivo* and *in vitro* study. In the present *in vivo* study, we used fear conditioning in mice to evaluate acute effects of X-irradiation and carbon ion irradiation on memory formation. Using 10-12 weeks old C57BL/6 male mice, single doses of 10 Gy of X-rays and carbon ion beam were administered to their whole brains. We then conducted fear conditioning training 7 hr and 24 hr after irradiation. Those mice were examined for context test 24 hr after training and for tone test 48 hr after training. We found that the mice irradiated by X-rays at 7 hr before training did not retrieve the contextual and auditory memory, but those at 24 hr before training retrieved the both memory. On the other hand, the mice irradiated by carbon ion beam at 7 and 24 hr before training did not seem to retrieve the contextual memory. For *in vitro* study we prepared mouse primary hippocampal cultured neuron and irradiated them with single dose of 10 Gy of X-rays or carbon ion beam at 21 DIV (days *in vitro*). For X-rays study we analyze the synaptic proteins; drebrin as post synaptic marker and Synapsin I as presynapstic marker. We found that the number of drebrin clusters significantly decreased at 8 hr and returned to the normal level at 24 hr, while Synapsin I did significantly decrease at 8 hr and 24 hr after irradiation. For carbon ion study, neurons were transfected with GFP to analyze the number of dendritic spines, and fixed at 8 hr and 24 hr after the irradiation and analyzed immunocytochemically. We found that the numbers of dendritic spines decreased significantly at 8 hr and 24 hr after irradiation. This indicates that the acute effects of X-irradiation on synaptic proteins are transient as well as fear memory formation, but those of carbon ion irradiation on dendritic spines remains at least within 24 hr. This study suggests that the acute effects of carbon ion irradiation seem to be more deteriorates to synaptic function than X-irradiation.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: US Army (W81XWH-12-0454)

Title: Effects of self-administered nicotine on fear conditioning in rats

Authors: C. WEBBER[1], C. W. ADAM[2], K. STOLL[2], E. G. MELONI[1], *S. B. CAINE[2], W. A. CARLEZON[1]
[1]Dept. of Psychiatry, Harvard Med. School, McLean Hosp., Belmont, MA; [2]ADARC, Mclean Hosp., BELMONT, MA

Abstract: Nicotine can reduce stress and improve coping. It can also enhance cognitive performance and alertness, and facilitate certain forms of learning. These two actions can be conceptualized as having opposite effects on vulnerability to develop post-traumatic stress disorder (PTSD). We designed experiments to examine how nicotine self-administration (SA) followed by a period of abstinence affected the development, expression, and persistence of PTSD-like symptoms as assessed in the fear-potentiated startle (FPS) paradigm. Exaggerated startle and resistance to extinction are observed in humans with PTSD, and these signs can be studied in animal models using FPS. Experimentally naïve Long-Evans rats were allowed to self-administer nicotine (0.03 mg/inj) or saline in 12-hr (overnight) extended access sessions in standard operant conditioning chambers for a minimum of 14 sessions. This amount of access was expected to produce nicotine dependence, determined by SA of >0.7 mg/session for 4 out of 5 sessions and observable signs of spontaneous withdrawal 11.5 hrs post SA session. After meeting these criteria, separate groups of rats (N=9-10/group) were fear-conditioned at either of 2 time points: immediately after or 12 hrs after their last SA session. Fear conditioning consisted of 10 pairings of a 3-sec light co-terminating with a 1-sec 0.6 mA footshock. After fear conditioning, SA sessions were discontinued. Percent FPS (%FPS) was quantified across 3 test sessions, 48 hrs apart, 10-12 days after fear conditioning and expressed as the percent change in startle on light + startle trials over startle alone trials. In rats fear conditioned immediately after the final SA session, there were no significant differences in %FPS over the 3 test sessions between rats that had self-administered nicotine or saline. However, during test session 1, nicotine-treated rats had lower responsiveness to startle alone than those treated with saline. In rats fear-conditioned 12 hrs after the last SA session, there were no differences in %FPS, but nicotine-treated rats had higher responsiveness to startle alone during test session 1. These findings may indicate that rats self-administering nicotine immediately prior to fear conditioning show signs of protection from exaggerated responses to the startle stimulus, whereas rats conditioned during nicotine withdrawal show signs of vulnerability. This work provides the basis for exploring the effects of nicotine SA on the development and persistence of fear in rats with continued access to nicotine after fear conditioning, and may ultimately provide deeper insight on how nicotine use affects vulnerability to stress-related illnesses.

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Poster

468. Fear and Aversive Memories: Modulation

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Support: NIH MH073949

Center for Translational and Basic Research (CTBR) at Hunter College

Yale University

Title: A curcumin-enriched diet prevents chronic corticosterone exposure from enhancing the consolidation and reconsolidation of a Pavlovian fear memory

Authors: *M. S. MONSEY[1], L. M. BOYLE[1], M. L. ZHANG[1], C. P. NGUYEN[1], D. M. GERHARD[1], J. R. TAYLOR[1,2,3], G. E. SCHAFE[4,5]
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Abstract: Chronic stress has been implicated in the development of post-traumatic stress disorder (PTSD), an anxiety disorder which is characterized by unusually strong and persistently reactivated fearful memories. We have recently shown that chronic exposure to the stress-associated adrenal steroid corticosterone (CORT) leads to a persistent enhancement in memory-related gene expression in the lateral amygdala and to enhanced consolidation of a Pavlovian fear memory. Here, we asked whether chronic CORT exposure can also enhance the reconsolidation of a Pavlovian fear memory. Further, we asked whether a diet enriched with curcumin (diferuloylmethane; derived from *Curcuma longa*) is capable of preventing CORT-related enhancements in fear memory consolidation and reconsolidation. Rats received chronic exposure to either water or CORT (50g/ml) in their drinking water for 2 weeks followed by an additional week of CORT titration (25g/ml for 3d, 12.5g/ml for 3d). During the 3-week CORT exposure period, half of the rats received a diet of standard rodent chow, while the other half received chow that was fortified with 1.5% curcumin to comprise 4 groups: 1) Water-regular chow, 2) Water-curcumin chow, 3) CORT-regular chow, 4) CORT-curcumin chow. All rats then received a 'wash-out' period for an additional 2 weeks consisting of water alone. In our consolidation experiment, rats were then fear conditioned with 2 pairings of a 5 kHz, 75 dB, 20 sec tone that co-terminated with a 1 sec, 0.5 mA footshock and tested for short-term memory (STM) and long-term memory (LTM) at 2 and 24 hr after fear conditioning, respectively. In our reconsolidation

experiment, rats were fear conditioned followed by a period of chronic CORT exposure with or without a curcumin-enriched diet. They then received a memory reactivation trial consisting of exposure to a single CS tone followed by tests of post-reactivation STM (PR-STM) and PR-LTM 2 and 24 hr later, respectively. Consistent with our previous findings, we observed that chronic CORT exposure enhances the consolidation of a Pavlovian fear memory; LTM is enhanced, while STM is unaffected. Further, rats with a history of chronic CORT exposure also exhibit enhanced fear memory reconsolidation; PR-LTM is enhanced, while PR-STM is not unaffected. Remarkably, each of these effects was prevented in rats fed a curcumin-enriched diet during the CORT exposure period. Collectively, our findings suggest that a history of chronic exposure to CORT can regulate fear memory consolidation and reconsolidation processes in a long-lasting manner and that a diet enriched with curcumin can prevent these effects.

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Poster

468. Fear and Aversive Memories: Modulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 468.05/UU92

Topic: F.02. Animal Cognition and Behavior

Title: Behavioral and cognitive alterations were not affected by fish supplementation in pilocarpine-induced temporal lobe epilepsy

Authors: *R. M. CYSNEIROS[1], F. A. SCORZA[2]
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Abstract: Temporal lobe epilepsy (TLE), the most common form of epilepsy, has substantial impact on behavior and cognitive process. Previously, we showed that fish oil (FO) affected hippocampal plasticity in pilocarpine-induced TLE (Ferrari, et al., 2008; Cysneiros et al., 2010). We studied the impact of FO supplementation in rats with epilepsy on learning, anxiety, and locomotion in a discriminative avoidance task. Wistar male rats at PN60 were subject to pilocarpine-induced epilepsy. Rats were divided into 4 groups: Control/Experimental groups treated with vehicle or fish oil (85mg/Kg/day) for 60 days. In the training session, the comparison between time spent into closed aversive arm (AVA) and non-aversive arm (NAVA) revealed significant effect of group [$F(1,44)=25.07$; $p<0.05$], of type of arms [$F(1,44)=373.89$;

p<0.05] and interaction F(1,44) =42.50; p<0.05] with no effect of treatment. All groups spent more time into closed NAVA as compared to AVA, but the time spent in AVA was significantly different between groups ([F(1,22)=10.06; p<0.05], regardless the treatment [F(1,22)=1.42; NS] with no effect of interaction between factors. Experimental rats spent more time in AVA comparatively to control group, suggesting learning deficit in a discriminative avoidance task. For the percentage the time spent on the open arms (%TOA), yield a significant effect of group [F(1,22)=21.67; p0.05] nor interaction between factors [F(1,22)=0.91; NS]. Experimental group spent more time in the OA, suggesting decreased level of anxiety. The total number of entries into arms (TENT) was significantly higher in experimental group [F(1,22)=14.61; p0.05] nor interaction between factors [F(1,22)=0.47; NS]. In the testing session, the %TAVA was a significantly higher in experimental group [F(1,22)=13.87; p<0.05], regardless the treatment [F(1,22)=1.77; NS] with no interaction between factors [F(1,22)=0.40; NS]. The % TOA was significantly higher in experimental group [F(1,22)=4.27; p<0.05], regardless the treatment [F(1,22)=0.01; NS] with no interaction between factors [F(1,22)=0.05; NS]. The total number of entries into arms was significantly higher in experimental group F(1,22)=11.40; p<0.05], with no effect of treatment [F(1,22)=0.06; NS] nor interaction between factors. Animals with epilepsy exhibited learning impairment, less level of anxiety and higher locomotor activity in a discriminative avoidance task. Fish oil supplementation did not affect any behavioral parameters investigated in this study. Sponsored by CNPq and FAPESP.

Disclosures: R.M. Cysneiros: None. **F.A. Scorza:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Program#/Poster#: 468.06/VV1

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NSERC

NARSAD

Title: Inhibition of mTOR kinase via rapamycin blocks persistent predator stress-induced fear memories

Authors: *J. J. BLUNDELL[1], J. WHITEMAN[2], K. FIFIELD[2], M. HEBERT[2]
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Abstract: Traumatic, stressful life events are thought to trigger acquired anxiety disorders such as post-traumatic stress disorder (PTSD). Recent data suggests that the mammalian target of rapamycin (mTOR) plays a key role in the formation of traumatic memories. The predator stress paradigm allows us to determine whether mTOR mediates the formation of both context-dependent (associative) and context-independent (non-associative) fear memories. Predator stress involves an acute, unprotected exposure of a rat to a cat which causes long-lasting non-associative fear memories manifested as generalized hyperarousal and increased anxiety-like behavior. Here, we show that rapamycin, an mTOR inhibitor, attenuates predator stress-induced hyperarousal, lasting at least three weeks. In addition, rapamycin blocks a subset of anxiety-like behaviors as measured in the elevated plus maze and hole board. Furthermore, when re-exposed to the predator stress context, rapamycin-treated stressed rats showed increased activity compared to vehicle controls suggesting that rapamycin blocks predator stress-induced associative fear memory. Interestingly, rapamycin's effect of predator stress-induced fear memories is time-dependent; it inhibits fear memories when given immediately around the time of the trauma but it potentiates fear memories when given 12-48 hrs post-trauma. Current studies are investigating the mTOR expression in key brain areas known to be involved in fear one hour and 1 week post-predator stress. Taken together with past research, our results indicate that mTOR regulation of protein translation is required for the formation of both associative and non-associative fear memories. Overall, these data suggest that mTOR activation may contribute to the development of acquired anxiety disorders such as PTSD.

Disclosures: J.J. Blundell: None. **J. Whiteman:** None. **M. Hebert:** None. **K. Fifield:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH Research Grant R01MH072672

NIH Training Grant T32NS082145

Title: Modeling cognitive therapy in the rat: Use of fear extinction training to reverse chronic stress-induced cognitive inflexibility and coping style choice

Authors: *E. A. FUCICH, J. DONEGAN, D. MORILAK
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Abstract: Stress-related mood and anxiety disorders, like depression or post-traumatic stress disorder (PTSD), are highly prevalent and lack effective treatment options for many patients. These disorders share a deficit in cognitive flexibility associated with prefrontal cortex dysfunction. Psychotherapies that invoke cognitive flexibility can be efficacious even in pharmacotherapy-resistant patients, though the neurobiological mechanisms underlying these effects are unknown. Pre-clinically, chronic unpredictable stress (CUS) causes a deficit in cognitive set-shifting, a measure of prefrontal cognitive flexibility using the attentional set-shifting test (AST), and also causes a shift from active to passive coping behavior in the shock-probe defensive burying (SPDB) test in rats. This study tested the hypothesis that the extinction of conditioned fear, which engages prefrontal cortical cognitive flexibility and closely resembles prolonged exposure therapy used for PTSD treatment, can model cognitive therapy in rats by improving performance in the AST and SPDB test after chronic stress. Results show that extinction training reversed CUS-induced deficits in extradimensional set-shifting and improved reversal learning, the two forms of cognitive flexibility measured with the AST. Also, extinction training appears to reverse the CUS-induced increase in immobility in SPDB. Western blot analysis of medial prefrontal cortex tissue collected after AST testing showed that chronic stress increased phosphorylation of the GluR1 receptor subunit at site S831, and extinction treatment normalized pGluR1(S831) to non-stress baseline levels. This study shows that fear extinction may effectively model a form of cognitive therapy, providing rationale for exploring its underlying mechanisms.

Disclosures: E.A. Fucich: None. **J. Donegan:** None. **D. Morilak:** None.

Poster

468. Fear and Aversive Memories: Modulation

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 468.08/VV3

Topic: F.02. Animal Cognition and Behavior

Title: Re-exposure to fear conditioned stimuli influences the relative use of learning strategy and enhances consolidation of habit memory

Authors: *K.-C. LEONG[1], M. G. PACKARD[2,1]
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Abstract: In a dual-solution plus maze task that is acquired using either hippocampus-dependent "place" or dorsolateral striatum-dependent "response" learning, robust emotional arousal produces a bias toward response learning. Studies from our laboratory employed "unconditioned" stimuli including injection of anxiogenic drugs or exposure to predator odor to induce emotional arousal and to modulate memory. In contrast, few studies have examined the effects of emotionally-arousing "conditioned" stimuli in modulating the relative use of multiple memory systems. Therefore, two experiments were designed to examine the ability of aversive conditioned stimuli to influence the relative use of learning strategies, and the consolidation of dorsolateral striatum-dependent habit memory. First, adult male Long-Evans rats received standard fear-conditioning trials in which a tone (2 kHz, 80 dB, 20 secs) was paired with a brief electrical shock (1 mA, 2 secs) three times. On subsequent days, different groups of rats received training in either a dual-solution water plus-maze task or a single-solution response water plus-maze task. In the dual-solution task rats were trained to swim from the same start arm (South) to a hidden escape platform that was always located in the same goal arm (East). In the single-solution task rats were started from varying start arms (North, South) and trained to always make the same body turn response (e.g. turn right) at the maze choice point. Immediately following water maze training, rats received post-training re-exposure to the conditioned aversive stimuli (i.e. tone and context that were previously paired with shock). The relative use of place or response learning was later assessed in the dual-solution task on a probe trial in which rats were started from the opposite start arm. On the probe trial, rats that swam to the spatial location in which the platform was located during training were designated place learners, whereas rats that made the body turn response reinforced during training were designated response learners. Post-training re-exposure to aversive conditioned stimuli produced preferential use of a response strategy in the dual-solution task. In addition, post-training re-exposure to aversive conditioned stimuli enhanced memory consolidation in the single-solution response learning task. These novel results indicate that exposure to aversive conditioned stimuli modulates the relative use of multiple memory systems in a manner similar to anxiogenic drug administration or exposure to predator odor, biasing rats toward the use of dorsolateral-striatal dependent habit learning, and enhancing consolidation of habit memory.

Disclosures: K. Leong: None. **M.G. Packard:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1R21MH086805

NIH Grant 1R01MH091147

Title: Role of social dominance in Pavlovian fear conditioning and the social transmission of fear

Authors: *C. E. JONES, M. H. MONFILS
Psychology, The Univ. of Texas, Austin, TX

Abstract: Most animal models of fear learning focus on creating a CS-US association through direct experience using variations of Pavlovian fear conditioning. We have previously shown that some rats will display a conditioned response (CR; e.g. freezing) to a cue after interacting with a cage-mate previously fear conditioned to a cue while this cage-mate is displaying the CR during the presentation of a cue (Bruchey et al., 2010; Jones et al., 2014). In the current study, we sought to further investigate the individual differences seen in social fear acquisition by controlling for dominance status of the rats undergoing this fear conditioning by-proxy (FCbP) paradigm. One rat from each cage of a triad was fear conditioned to a tone CS. The next day, the conditioned rat was returned to the chamber accompanied by a second cage-mate while the tone was played in the absence of the foot-shock (FCbP). Socially acquired fear was measured as freezing displayed by this second cage-mate to the CS alone on the following day. Social dominance in male rats was determined using the methods of Pellis et al. (1993) by observing and characterizing play behavior amongst a triad of related adults. We then manipulated which rat of the hierarchy was directly fear conditioned or fear conditioned by-proxy. Our results show that the two subordinate rats tend to have an unequal relationship with the dominant and this relationship can predict the amount of fear acquired "by proxy" with subordinate rats freezing more after observing and interacting with the dominant than the dominant rat after observing and interacting with a subordinate. We also found that the amount of reciprocal play behavior observed within two rats is predictive of the level of fear transmitted socially when those rats are fear conditioned in the FCbP paradigm. We further show that social dominance status can influence conditioned fear responses to context and investigate its role in testosterone and corticosterone levels in serum.

Disclosures: C.E. Jones: None. **M.H. Monfils:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 468.10/VV5

Topic: F.02. Animal Cognition and Behavior

Support: R01MH091147

Title: Fear conditioning at p17/p25: The effects of fluoxetine on retention and re-conditioning in adulthood

Authors: *R. ROQUET[1], E. BAUER[2], M. MONFILS[1]
[1]Psychology, The Univ. of Texas At Austin, Austin, TX; [2]Biol. Sci., Barnard Col., New York, NY

Abstract: Early life experiences shape development. Importantly, adverse early life experiences can lead to vulnerabilities in mood and anxiety-related disorders. Evidence from our lab and others' suggests that fear conditioning leads to different behavioral effects depending on the age at which it occurs. For example, after fear conditioning on postnatal day 17 (P17), rats show no difference in freezing behavior relative to naïve controls when presented with the conditioned stimulus (CS) in adulthood. However, if rats are conditioned a few days later on postnatal day 25 (P25), they show increased levels of freezing over controls when tested in adulthood. This difference in freezing behavior during the initial retention test does not show the same pattern after re-conditioning, however, in that both P17 and P25 rats re-conditioned in adulthood show an exacerbated freezing response when tested for long-term memory (LTM). Here, we conditioned rats at either P17 or P25, then tested the effects of serotonin reuptake inhibitor (SSRI; Fluoxetine [Prozac]) or saline, administered chronically in adolescence, on fear retention and re-conditioning in adulthood. Prozac is proposed as a possible intervention because it is both widely prescribed, and currently the only SSRI approved for children and adolescents, yet has also been shown to promote hippocampal neurogenesis, in addition to anxiolytic properties in rodent models of anxiety-like behavior (Emslie et al., 2002; Santarelli et al., 2003). Our data show that, independent of adolescent treatment, rats conditioned at P25 show retention and increased freezing to the first CS presented in adulthood relative to controls. In P17 rats, there is a trend for an interaction of increased freezing to the first CS in early life conditioned rats that received Prozac. Specifically, rats conditioned at P17 that did not receive Prozac showed no retention of fear in adulthood, but those that received Prozac showed increased freezing. Our results further show that when tested post conditioning in adulthood, rats froze to equivalent levels regardless of whether they had been conditioned at P25 and/or had received Prozac/Saline. For the P17 groups, all rats that were conditioned in adulthood, regardless of early experience and/or Prozac/Saline, froze significantly more during LTM than the rats that were not conditioned in adulthood. Our results may indicate that (1), chronic fluoxetine treatment after adverse experience in early life exacerbates fear memory and expression in rats conditioned at P17, or that (2) chronic fluoxetine treatment may promote the recovery of explicit memories.

Disclosures: **R. Roquet:** None. **E. Bauer:** None. **M. Monfils:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Program#/Poster#: 468.11/VV6

Topic: F.02. Animal Cognition and Behavior

Support: 1R01MH091147

1R21MH086805

Title: Reconsolidation update mechanisms and metabolic enhancer USP methylene blue operate independently to attenuate fear memories

Authors: ***A. AUCHTER**, F. GONZALEZ-LIMA, M. H. MONFILS
Psychology, Univ. of Texas At Austin, Austin, TX

Abstract: For decades, we have known that extinction of conditioned fear does not lead to erasure of the fear memory. However, we have combined the principles of memory reconsolidation and extinction to show that extinction training administered after an isolated retrieval prevents the return of fear more persistently than extinction alone. Methylene blue USP (MB) is a neurometabolic enhancer shown previously to be effective in enhancing fear extinction memory consolidation. The purpose of the present study was to develop a predictive model for minimizing the return of fear using the retrieval/extinction paradigm in conjunction with MB. Using a 2x2 factorial design, we predicted that both presentation of a retrieval CS (vs. no retrieval CS) before extinction and administration of MB (vs. saline) post-extinction would enhance the retention of the extinguished conditioned response. Due to MB's consolidation-enhancing effect, we also predicted that the better subjects extinguished conditioned fear within session (i.e. showed a large decrease in freezing from the beginning to the end of extinction), the more likely they would form a memory that the CS is less threatening, and thus the more they would benefit from post-extinction administration of MB. Indeed, in subjects that received post-extinction treatment with MB, level of freezing at the end of extinction predicted the amount of fear that returned after reinstatement of the US. In subjects that received saline, post-reinstatement return of fear was not predicted by level of freezing at the end of extinction. As expected, subjects that received the retrieval CS showed less post-reinstatement return of fear. Interestingly, this effect was independent of MB treatment. This indicates that the reduction of

fear as a result of the isolated retrieval could not be explained by strengthened extinction alone. Overall, these findings indicate that post-extinction administration of MB helped consolidate the memory for extinction, ensuring that subjects remained at post-extinction levels of freezing. An isolated retrieval followed by extinction resulted in more persistent fear reduction independently of MB, further implicating destabilization and memory updating in the retrieval/extinction paradigm.

Disclosures: **A. Auchter:** None. **F. Gonzalez-Lima:** None. **M.H. Monfils:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: Fapesp

Title: Sodium Nitroprusside improves the fear conditioning deficit in a schizophrenia animal model - the SHR strain

Authors: ***M. C. DIANA**, F. F. FIEL, M. A. SUIAMA, V. V. O. JUSTI, N. D. DA SILVA, V. C. ABÍLIO
UNIFESP, São Paulo, Brazil

Abstract: Schizophrenia is one of the most severe and disabling mental disorders. Its treatment is performed with antipsychotics drugs. However, these drugs are not able to benefit all patients and often cause devastating side effects. In this context, it is of great interest to investigate new drugs and therapeutic approaches, not only for the treatment of the disease as well as for its prevention. Early evidence has emerged supporting the role of the nitrergic system in the pathophysiology of schizophrenia. Moreover, recent data show a striking and lasting therapeutic effect of a nitric oxide donor - sodium nitroprusside - in the treatment of refractory patients. It's noteworthy that literature data are controversial: while some researches advocate for an increase of nitric oxide in schizophrenic patients, others show diminished levels of this molecule. Alongside, studies in animal models of schizophrenia demonstrated beneficial effects of both nitric oxide donors and inhibitors. Recently our group has found that the Spontaneously Hypertensive Rat (SHR) strain presents a schizophrenia behavioral phenotype which is specific reversed by antipsychotic drugs and aggravated by proschizophrenia manipulations. Therefore,

the aim of this study was to evaluate the effect of a nitric oxide donor - sodium nitroprusside (SNP) - in the fear conditioning (used to assess emotional processing) deficit of the SHR strain. Three dosages of SNP (0.5; 2.5; 5.0 mg/kg) were injected in SHR animals and Wistar rats (control strain). 24 hours later these animals were submitted to the training session of the contextual fear conditioning test (footshocks are associated to a neutral environment). 24 hours later the animals were tested and freezing behavior (fear response indicative of the aversive conditioning between the shock and the environment) was quantified. As expected, the SHR strain presented deficits in freezing behavior compared to control rats. Treatment with 5.0 mg/kg SNP was able to improve the fear conditioning deficit of SHRs. These results demonstrate an antipsychotic potential of SNP for aversive emotional memory and reinforces its potential use in schizophrenia patients.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant GM103643

NIH Grant MH093950

Title: Faah inhibitor ol-135 disrupts contextual, but not auditory, fear conditioning in rats

Authors: *K. SZOLUSHA[1], R. BIND[2], K. KERNEY[2], D. BOGER[4], E. BILSKY[2], M. BURMAN[3,2]
[2]Ctr. for Excellence in the Neurosciences, [3]Psychology, [1]Univ. of New England, Biddeford, ME; [4]Chem., The Scripps Res. Inst., La Jolla, CA

Abstract: Anxiety disorders are the most common psychological disorder, with an approximately 25% lifetime incidence. The endocannabinoid system has increasingly been the target of investigation for a potential role in fear and anxiety. Research on compounds such as CB1 agonists and antagonists have demonstrated effects on fear and anxiety using both innate anxiety-like behaviors and conditioned fear. However, direct CB1 agonists and antagonists often

have undesirable cognitive side effects. Fatty acid amide hydrolase (FAAH) is a degradation enzyme targeting the endocannabiniods. Disrupting FAAH avoids many of the deleterious effects of direct CB1 manipulation. Interestingly, drugs that inhibit FAAH have two major effects: they enhance memory formation and they inhibit innate measures of anxiety. They don't appear to have been assessed in classical fear conditioning, where these two effects would appear to compete. The current studies utilize classical fear conditioning, a particularly successful model of fear/anxiety, in which a previously neutral cue is associated with an aversive stimulus, such that the neutral cue comes to elicit a conditional fear response. In addition to fear for the explicitly conditioned cue (auditory fear), fear also develops to the conditioning apparatus and situation (contextual fear conditioning). These two tasks also require different neural substrates, in that fearing an auditory cue requires the amygdala and its efferent and afferent connections, whereas contextual fear conditioning recruits additional hippocampal and cortical circuitry. To examine the role that endocannabiniods play in fear conditioning, we injected the FAAH inhibitor OL-135 at 5.6 mg/kg and 10 mg/kg both before and after fear conditioning. We then assessed both auditory and contextual fear. When the FAAH inhibitor was injected before conditioning there was a specific deficit in contextual fear. That auditory fear expression was intact and there was no difference in the shock reactivity of the animals suggests that these results are not due to analgesic or non-specific effects of the drug. When the animals were injected after training there was no effect, suggesting the endocannabinoid system is only required during acquisition of fear. These data are consistent with previous research showing that CB1 manipulation causes deficits of acquisition, but not consolidation, of fearful memories. These data further the promise of these compounds for the treatment of fear and anxiety.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

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NRF Grant GPF-2011

Title: Memory recall and modifications by activating neurons with elevated CREB

Authors: J.-T. KWON[1], J. KIM[1], H.-S. KIM[1], S. A. JOSSELYN[2], *J.-H. HAN[1]
[1]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of; [2]Hop. for Sick Children, Toronto, ON, Canada

Abstract: Memory is thought to be supported by a specific set of neurons throughout multiple brain regions, forming unique memory trace. Reactivation of these neurons may elicit behavioral expression of that memory and also affect the strength or stability of the memory. However, the causal link remains elusive. We have shown previously that neurons in lateral amygdala (LA) with increased level of cyclic adenosine monophosphate response-element binding protein (CREB) are essential for fear memory expression, identifying critical neurons within fear memory trace. Here we used ectopic rat vanilloid receptor TRPV1/capsaicin system to selectively activate these neurons. Activating these neurons after learning induced significant freezing response, indicating recall of fear memory. This process returned stabilized fear memory labile state but repeated activation strengthened the memory. Therefore, our findings identify essential ensemble of neurons in memory trace whose activation is sufficient for memory recall and its dynamic changes.

Disclosures: J. Kwon: None. **J. Han:** None. **J. Kim:** None. **H. Kim:** None. **S.A. Josselyn:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NRF Grant No.2011-0013173

NRF Grant GPF-2011

Title: Optogenetic activation of presynaptic inputs in lateral amygdala forms associative fear memory

Authors: *J. KWON[1], R. NAKAGIMA[2], H.-S. KIM[1,2], Y. JEONG[1], G. AUGUSTINE[2], J.-H. HAN[1]
[1]Biol. science, KAIST, Daejeon, Korea, Republic of; [2]KIST, Seoul, Korea, Republic of

Abstract: In Pavlovian fear conditioning, the lateral amygdala (LA) has been highlighted as a key brain site for association between sensory cues and aversive stimuli. However, learning-related changes are also found in upstream brain regions such as sensory thalamic and cortical areas. If the synaptic connections between sensory inputs and LA neurons are the actual sites of fear learning, then direct activation of presynaptic sensory inputs should be sufficient to form fear memory. To test this prediction, we used optogenetics to selectively photostimulate presynaptic auditory inputs that project to the LA. Channelrhodopsin was expressed in the two main auditory input pathways to LA, the medial geniculate nucleus of the thalamus and the secondary auditory cortex, allowing their axonal projections in the LA to be photostimulated as a conditioned stimulus (CS). Pairing this CS with aversive foot-shock, which served as an unconditioned stimulus, caused the CS to produce robust freezing twenty-four hours later, demonstrating that activity in the presynaptic auditory inputs to the LA alone was sufficient to serve as a CS for formation of a fear memory trace and long-term fear memory. Our results prove for the first time that sensory input-to-LA synapses are sufficient to serve as a CS, thereby isolating a very specific brain site for the formation of an associative fear memory and providing compelling evidence that associative fear learning occurs at this site. Our study also suggests that, within the broader circuitry involved in fear, there are key circuit components that can support a given fear memory.

Disclosures: J. Kwon: None. **H. Kim:** None. **Y. Jeong:** None. **J. Han:** None. **R. Nakagima:** None. **G. Augustine:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: ERC-STG-337747

FCT:SFRH / BD / 51257 / 2010

BIAL:178/10

Title: Oxytocin in the central nucleus of the amygdala gates freezing allowing for maternal defense responses and transmission of fear to offspring

Authors: *E. RICKENBACHER[1], R. PERRY[2,3,4], K. SZYBA[2], S. AL AIN[2,3,4], R. SULLIVAN[2,3,4], M. MOITA[1]
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Abstract: It is well established that mothers become more defensive around parturition. For instance, female rats become aggressive towards unfamiliar males, potential predators to the pups, and show less anxiety and passive fear responses compared to virgin females. It however remains to be established whether the presence of offspring modulates the fear response of mothers. To address this issue, we used fear conditioning, a well-known paradigm for which the neural circuit is described. We conditioned female rats to fear an odor, and re-exposed them to the conditioned odor, either in the presence or absence of pups. We found that mothers exposed alone froze to the odor, but mothers in the presence of their pups did not. We postulated that pup presence leads to the release of oxytocin (OXT) in the Central Nucleus of the Amygdala (CeA), where it is known to inhibit output neurons that drive freezing. To test the role of OXT in gating freezing, we infused an antagonist of OXT or vehicle into the CeA of mothers exposed to the conditioned odor in the presence of their pups. Mothers, which received the antagonist infusion, showed strong freezing even in the presence of their pups, whereas mothers with vehicle infusions did not. Finally, it is well established that offspring learn about environmental factors through the responses of the mother. The transmission of fear of the odor from mother to pup was examined in PN 19-21 pups. Pups from both the oxytocin and vehicle infused mothers were re-exposed to the odor in the absence of their mother at post weaning age PN 23-25. Pups which had been exposed to the odor in the presence of the vehicle infused conditioned mothers froze to the odor when tested alone. However, pups which had been in the presence of mothers infused with OXT froze significantly less. As OXT antagonist mothers froze, instead of interacting with their pups, we hypothesize that proximity to the pup during exposure to the odor is necessary for the transmission of fear to the pup. This work clearly demonstrates that oxytocin in the CeA post partum, blocks freezing allowing maternal defense behaviors during exposure to a conditioned cue. Additionally, maternal behaviors triggered by a fearful stimulus are essential for the transmission of fear information from the mother to the offspring.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

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Title: Molecular mechanisms of VNS-enhanced extinction of fear

Authors: ***A. ALVAREZ-DIEPPA**, J. CHILDS, S. WILLETT, S. KROENER, C. MCINTYRE
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Abstract: Administration of vagus nerve stimulation (VNS) during trials of fear extinction reduces expression of conditioned fear faster than extinction training alone. High frequency stimulation administered to the infralimbic area of the prefrontal cortex (IL) 24 hours after behavioral testing produces long-term potentiation (LTP) in the basolateral complex of the amygdala (BLA) in VNS-treated rats, whereas Sham-treated rats did not demonstrate measurable extinction learning and showed long term depression (LTD) in the BLA. The present study was designed to elucidate molecular mechanisms by which VNS expedites extinction of conditioned fear and promotes LTP in the BLA. The calcium/calmodulin-dependent protein kinase (CaMKII) is a synaptic protein that undergoes autophosphorylation at Thr286, and plays an important role in induction and maintenance of LTP by interacting with both AMPA and NMDA receptors. The activity-regulated cytoskeletal-associated protein (Arc) is involved in LTD via its role in AMPA receptor-related synaptic changes. Male Sprague-Dawley rats were subjected to 2 days of auditory fear conditioning followed by extinction training paired with VNS or Sham stimulation 24 hours later. Two groups were added to the experiment for comparison: rats that underwent fear conditioning alone (FC), and rats that were not given VNS but that reached successful extinction after receiving extra exposures to the tone (EE). Western blots were used to quantify expression of CaMKII and Arc proteins, and to assess possible changes in receptor activity by measuring phosphorylation of the GluA1 subunit of AMPA receptors, and by quantifying expression of the GluN2A and GluN2B subunits of NMDA receptors after behavioral treatment. Rats given VNS during extinction training and EE rats showed a significant increase in phosphorylation of CaMKII at Thr286 and GluN2B expression, as compared to Sham and FC rats. Arc expression was significantly reduced in VNS-treated rats compared to all groups. No significant differences were seen in total levels of CaMKIIα or CaMKIIβ, or in GluA1 or GluN2A expression across groups. Results suggest that VNS-enhanced extinction reverses the disposition to LTD and promotes LTP in the IL-BLA pathway by reducing fear-conditioning associated Arc expression, and promoting CaMKII-induced GluN2B expression in the BLA.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: HHMI grant to Swarthmore College

Title: Learning to unlearn fear: A novel animal model of exposure therapy

Authors: ***A. M. SCHNEIDER**[1], P. E. SIMSON[2], D. KALAMARIDES[1], M. LICHTEN[1], J. WALSH[1], C. DAIMON[1], L. G. KIRBY[3]
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Abstract: The effect of extinction in laboratory animals, like the effect of exposure therapy in humans, is temporary and with passage of time fear often recovers. In a recent experiment we used a modified extinction procedure in which, 24 hr after contextual fear conditioning, animals were returned to the apparatus in the absence of shock. In contrast to the typical extinction procedure, the return was brief and the animals were removed from the apparatus before they had an opportunity to extinguish the fear, that is, they were removed either at the onset of weak (incipient) fear (30-sec exposure) or at the peak of intense (incubated) fear (120-sec exposure). A retention test revealed that the 30-sec exposure, not the 120-sec exposure, was effective in reducing retention of fear. To account for these results we suggest that the brief (30 sec) exposure, in addition to initiating retrieval of fear, itself serves as a learning experience (that is, during the brief exposure the conditioning chamber may be associated with retrieved fear). Thus, because retrieved fear during the 30-sec exposure is relatively weak, the 30-sec exposure results in weak conditioning and weak retention the next day; because retrieved fear during the 120-sec exposure is relatively strong, the 120-sec exposure results in enhanced conditioning and strong retention. To test this hypothesis, in the present study, we added a second 30-sec exposure to the first: If the brief exposure procedure indeed serves as a learning experience, then adding the second 30-sec exposure should increase the effectiveness of the procedure in reducing retention of fear. Male Long-Evans rats underwent contextual fear conditioning followed 24 hr later by the

brief exposure procedure; a retention test was administered either 1 or 13 days later. Fear conditioning consisted of placing rats in a dark compartment for 120 sec followed by a single shock (0.8 mA, 0.5 sec); the brief exposure procedure consisted of confining the animals to the dark compartment for either a single 30-sec exposure or for two 30-sec exposures separated by 10 min; the retention test consisted of returning the animals to dark compartment for 180 sec. The results indicated that the additional 30-sec exposure increased the effectiveness of the procedure in reducing retention of fear both in the short term (24 hr) and the long term (13 days). Thus, the present study suggests that, in contrast to the typical extinction procedure, the brief exposure procedure may be more effective in limiting recovery of fear. Viewed from a therapeutic perspective, these results have implications for the efficacy of a modified exposure therapy protocol in the treatment of pathological fear memory.

Disclosures: A.M. Schneider: None. **P.E. Simson:** None. **D. Kalamarides:** None. **M. Lichten:** None. **J. Walsh:** None. **C. Daimon:** None. **L.G. Kirby:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Fundação Champalimaud

ERC-STG-337747

Title: Been there, done that: Experiencing freezing is required for observational fear in the context of social interactions

Authors: *A. CRUZ, M. A. MOITA
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Abstract: It has been shown that rats respond to the display of defensive responses by con-specifics. Furthermore, rats with prior experience with shock show observational freezing but naïve ones do not. We hypothesized that, during exposure to shock, rats associate their own defense responses, such as freezing, with aversive stimuli. Thus, freezing would become an alarm cue through its association with shock. Exposure to shock may also contribute to

observational freezing through stress-induced sensitization. To test whether associative learning underlies freezing during a social interaction, we tested different exposures to shock. We found that experiencing shock alone is not sufficient, as animals that receive immediate shocks in an unfamiliar context do not display observational fear. This protocol entails no freezing nor fear learning. To test if fear learning in the absence of freezing is sufficient to sustain transmission of fear, we conditioned rats to fear a context without expressing fear responses in that environment. We found that these rats show low levels of observational fear. These results support our hypothesis that the animal has to associate it's own fear responses with shock. To rule out stress induced sensitization we compared corticosterone levels in two shock conditions and found differences in the strength of the stress response. Hence, we subjected observers to a different kind of stress using forced swim. We found that these animals do not display observational freezing. Previous work from our lab found that the transition from sound to silence is both necessary and sufficient for transmission of fear. Hence, we hypothesized that the silence generated from freezing during prior experience could become an alarm cue via its association with shock. To test this we conditioned animals with the sound of a moving rat in the background. We found that these animals freeze during both conditioning and the social interaction.

Disclosures: **A. Cruz:** None. **M.A. Moita:** None.

Poster

468. Fear and Aversive Memories: Modulation

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SFRH/BD/33943/2009

Bial Fellowship 178/10

Champalimaud Foundation

Title: Fearful silence: The role of lateral amygdala and its auditory inputs in social transmission of fear

Authors: *A. PEREIRA, S. Q. LIMA, M. A. MOITA
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Abstract: It is well documented that social information can be used to signal danger. We previously developed a behavioral paradigm to study transmission of fear between rats, and found that rats perceive the cessation of movement-evoked sound as a signal of danger and its resumption as a signal of safety. The Lateral Amygdala (LA) is a structure widely implicated in fear responses, receiving auditory inputs from both cortical and thalamic pathways. Therefore, we hypothesize that the cessation of movement-evoked sound leads to activation of auditory inputs to LA switching on freezing. We have started using optogenetic tools to inactivate LA specifically during the transitions from movement-evoked sound to silence. Our preliminary results suggest that inactivation of LA during this transition disrupts the expression of freezing during the periods of silence. In addition, to identify the auditory input areas involved in the detection of silence onset, we used the expression of c-fos, an immediate early gene, as neural activity markers. Rats were exposed to either a continuous playback of movement-evoked sound or the same playback with two periods of silence (to which rats freeze). We predicted that areas whose activity is driven by the cession of movement-evoked sound would show increased number of neurons expressing c-fos in the group exposed to the sound playback with two periods of silence relative to the group exposed to continuous sound. We have focused on auditory thalamus sub-regions which project both to cortex and directly to LA. We found an increase in c-fos expression exclusively in the dorsal Medial Geniculate Nucleus (MGd) . This is in accordance with an earlier study showing that MGd is a sub-region of the auditory thalamus with the highest number of cells with white noise offset responses, suggesting that these neurons might signal the cessation of movement-evoked sounds. This study will contribute to our current understanding of the neural mechanisms of fear, by providing information on how fear can be regulated by natural sounds.

Disclosures: A. Pereira: None. **S.Q. Lima:** None. **M.A. Moita:** None.

Poster

468. Fear and Aversive Memories: Modulation

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Program#/Poster#: 468.21/VV16

Topic: F.02. Animal Cognition and Behavior

Title: Enhanced consolidation of habit memory by post-training exposure to a fear CS is blocked by propranolol administration

Authors: *J. GOODMAN[1], K.-C. LEONG[2], T. D. GOODE[3], S. MAREN[3], M. G. PACKARD[3]
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Abstract: Previous studies from our laboratory have employed unconditioned stimuli including injection of anxiogenic drugs or exposure to predator odor to induce emotional arousal and enhance dorsolateral striatum-dependent habit memory. In addition, we have recently observed that re-exposure to aversive conditioned stimuli (CS) (i.e., a tone and context that were previously paired with shock) can also enhance consolidation of dorsal striatum-dependent memory. In the present experiments we examined the effect of the beta-adrenoceptor antagonist propranolol on the memory enhancement induced by re-exposure to fear-related conditioned stimuli. First, adult male Long-Evans rats were trained in a standard fear-conditioning paradigm in which a tone (2kHz, 80dB, 20s) was paired with a shock (1mA, 2s) three times. On subsequent days, the rats were trained in a dorsolateral striatum-dependent water plus-maze task to swim from alternating start points (north, south) and make the same body turn response (e.g., turn right) at the choice point to reach a hidden escape platform (6 trials/day for 5 days). Immediately following training on days 1-3, rats received post-training peripheral administration of propranolol (3 mg/kg, i.p.) or saline vehicle and were then re-exposed to the previously conditioned tone and context without shock. Post-training re-exposure to the fear CS enhanced consolidation of habit memory in vehicle-treated rats, and this effect was blocked by propranolol administration. Overall, the findings indicate that the enhancement of dorsal striatum-dependent memory consolidation engendered by conditioned fear may involve the noradrenergic system.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH MH073949

Center for Translational and Basic Research (CTBR) at Hunter College

Title: Effects of fluoxetine on molecular correlates of pavlovian fear conditioning in rats with a history of chronic corticosterone exposure

Authors: *M. A. BRIONES[1,2], M. S. MONSEY[3], L. M. BOYLE[3], M. L. ZHANG[3], C. P. NGUYEN[3], M. SELIGSOHN[2], T. K. WINER[2], K. LOPEZ[2], G. E. SCHAFE[2,1]
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Abstract: Chronic stress has been strongly implicated in the development of post-traumatic stress disorder (PTSD), an anxiety disorder which is characterized by unusually strong and persistently reactivated fearful memories. We have recently observed that chronic exposure to the stress-associated adrenal steroid corticosterone (CORT) enhances the consolidation of a Pavlovian fear memory, an effect which can be reversed by treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX). Here, we examined the effects of chronic CORT exposure and chronic CORT exposure followed by FLX on several key molecular correlates of auditory Pavlovian fear conditioning in the lateral amygdala (LA). Rats received chronic exposure to either plain water or CORT (50 μg/ml) in their drinking water for 2 weeks, followed by an additional week of CORT titration (25 μg/ml for 3 days, 12.5 μg/ml for 3 days). Rats then received a 'wash-out' period for an additional 3 weeks during which they were exposed to either water alone or 333 μg/ml FLX in their drinking water to comprise 4 groups: Water/Water, Water/FLX, CORT/Water or CORT/FLX. We observed that chronic exposure to CORT leads to a persistent upregulation in the expression of the glutamatergic subunit GluR1 and the excitatory amino acid transporter vGluT in the LA, effects which were reversed by treatment with FLX. In fear conditioning experiments, we observed that auditory Pavlovian fear conditioning leads to an increase in ERK1/2 phosphorylation and the expression of the immediate early genes (IEGs) Arc/Arg3.1 and Egr-1 in the LA, effects which were further enhanced in rats with a history of chronic CORT exposure. Further, treatment with FLX was observed to reverse these CORT-related enhancements in ERK1/2 activation and memory-related IEG expression in the LA. Treatment with FLX alone had no effect on the baseline expression of any of the proteins we examined in the LA. Our findings suggest that a history of chronic exposure to CORT can regulate glutamatergic signaling and associated downstream molecular pathways in LA neurons, effects that can be effectively reversed by treatment with FLX.

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Poster

468. Fear and Aversive Memories: Modulation

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Title: Effects of intra-amygdalar microinjection of the metabotropic group II (mGlu II) antagonist, LY341495 (LY34) on fear extinction and sleep

Authors: *L. L. WELLMAN, E. DONG, L. YANG, L. D. SANFORD
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Abstract: Metabotropic glutamate (mGlu) receptors consist of eight known receptor subtypes that have been categorized into Groups I, II, and III on the basis of sequence homology, second messenger coupling, and pharmacology. There has been significant interest in mGlu agents as therapeutics for psychiatric disorders and a variety of animal studies indicate a role for mGlu receptors in the basolateral nucleus of the amygdala (BLA) in regulating fear memory and extinction. mGlu receptors are also implicated in the regulation of sleep and we found that microinjections into BLA of the mGlu II agonist, LY379268 (LY37), selectively reduced REM without significantly altering wakefulness or NREM whereas microinjections of even high dosages of the mGlu II antagonist, LY341495 (LY34), into BLA suppressed NREM and total sleep, but did not significantly alter REM. By comparison, microinjection of either LY37 or LY34 into the central nucleus of the amygdala did not significantly alter sleep, indicating specificity for effects to BLA. In this study, we examined the effects of LY34 in BLA on fear extinction and on modulating the relationship between fear extinction and sleep. To conduct the study, two groups of rats were implanted with electrodes for recording sleep and with cannulae for bilateral microinjections into BLA. After recovery and habituation to handling procedures, the rats were trained with inescapable shock (20 footshocks, 0.8 mA, 0.5 sec duration, 1 min inter-stimulus interval). The next day, the rats received microinjections of vehicle (n=5) or LY34 (n=5) into BLA prior to a 60 min fear extinction trial. The concentration of LY34 was 30 nM, a dosage that did not significantly alter sleep when microinjected into BLA prior to undisturbed recording. Sleep was recorded and examined after handling control, after shock, after extinction and after a re-test day 8 days post-extinction. Freezing was examined during extinction and during the retest day. LY34 facilitated reduction in fear behavior during the second half of the extinction period and enhanced subsequent REM. LY34 prior to extinction was also associated with reduced freezing and enhanced REM on the subsequent re-test day. NREM was not altered

across conditions. By comparison, rats treated with vehicle showed a normalization of REM after extinction, as we previously reported for non-injected rats. These data demonstrate that mGlu II receptors in BLA modulate fear extinction and that a single administration of LY34 into BLA can have persisting effects on fear behavior and the effects of fear memory on REM.

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Poster

468. Fear and Aversive Memories: Modulation

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NARSAD and CNPq (Ciência sem Fronteiras)

Title: Adenosine A2A receptors in the amygdala control synaptic plasticity and conditional fear memory

Authors: *A. SIMÕES[1], N. GONÇALVES[2], D. RIAL[2], R. CUNHA[2,3]
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Abstract: The amygdala is primarily involved in processing emotional memory and displays enhanced synaptic transmission upon fear and depressive-like conditions. Notably, the blockade of adenosine A2A receptors (A2AR) with selective (e.g. SCH58261) or non-selective (e.g. caffeine) antagonists ameliorates stress-induced behavioral and neurochemical alterations. However, since the role of A2AR in the amygdala is unknown, we now studied the impact of A2AR in amygdalar synaptic transmission and in contextual fear. Using horizontal brain slices

from male c57bl6 mice (8-10 weeks), electrophysiological recordings in the lateral amygdala upon stimulation of cortical afferents showed that two selective A2AR antagonists decreased the amplitude of long-term potentiation (LTP) triggered by a high frequency train (121.9±3.5% with 50nM SCH58261 versus 148.6±7.9% in control, n=5-6, $p<0.05$ and 112.4±13.8% with 50nM ZM241385 versus 160.9±12.1% in control, n=4-5, $p<0.05$, respectively) while having no effect on basal transmission or on input-output curves. Inhibiting ecto-5'-nucleotidase (using its inhibitor α,β-methylene-ADP, AOPCP) with the concomitant blockade of ATP receptors (with the general antagonist PPADS) also abolished LTP (107.0±8.7% with 20μM PPADS and 100μM AOPCP versus 139.3±5.8% in control, n=4-5, $p<0.05$), indicating that ATP-derived adenosine activates A2AR controlling LTP. Finally, the bilateral injection of a lentivirus expressing an shRNA to down-regulate A2AR in the lateral amygdala decreased the expression of contextual fear memory (30.2±5.8s of freezing in the control group versus 13.8±3.5s of freezing in the shA2A group, n=5, $p<0.05$) 8 days after its acquisition (3 trials, separated by 2 minutes, each consisting of a tone cue paired with a co-terminating 2 second foot shock, begun after a 3 minute habituation period). Together, our results show that A2AR are important controllers of plastic adaptive changes in amygdalar circuits and A2AR emerge as promising target to treat mood disorders such as depression and post-traumatic stress.

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Poster

468. Fear and Aversive Memories: Modulation

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Support: VA merit grant (RS) 1I01BX001075

Title: Lentiviral-mediated overexpression of Neuropeptide Y in fear regulatory circuits: Neuroanatomical and behavioral characterization

Authors: ***S. SCHMELTZER**[1,3,2], L. L. VOLLMER[2], C. M. DOLGAS[2], T. D. GERACIOTI[3,2], S. P. WILSON[4], R. SAH[1,3,2]
[1]Neurosci. Grad. Program, [2]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH; [3]VA Med. Ctr., Cincinnati, OH; [4]Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Neuropeptide Y (NPY) is a 36 amino acid peptide transmitter that is abundant in forebrain limbic regions that regulate stress, anxiety, and fear. Evidence from preclinical and human studies demonstrate a role for NPY in stress coping, conferring that it may be a potential "resilience-to-stress" factor. We reported a significant reduction in cerebrospinal fluid (csf) NPY in combat-related PTSD subjects that shows significant correlation with the re-experiencing subscale of the Clinician Administered PTSD Scale (CAPS) score. This suggests that NPY may play an important role in the modulation of fear memories in disorders of emotional learning such as PTSD. In the current study we investigated the effects of NPY manipulation in the amygdala, a primary fear regulatory region that is implicated in PTSD. NPY delivered into the basolateral amygdala (BLA) prior to extinction training led to reduced expression of consolidated fear as well as improved extinction. We then used a lentivirus construct designed for NPY overexpression under the control of the phosphoglycerate kinase-1(PGK) promoter (SPWG-rNPYF). *In vitro* transfection of virus into 1V B cells led to a significant increase in the expression of NPY mRNA that was not observed in cells transfected with the GFP control construct. For *in vivo* characterization, SPWG-rNPYF was administered in the basolateral amygdala (BLA). Ongoing studies are investigating effects of BLA targeted SPWG-rNPYF on a) expression of NPY, NPY receptors, GAD65 and CAMKII in fear regulatory circuits b) anxiety and fear memory related behaviors and c) behavioral responses in an animal model of PTSD. Viral-mediated overexpression of NPY in a region-selective manner will lead to an increased mechanistic understanding of NPY contributions to PTSD pathophysiology.

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Poster

468. Fear and Aversive Memories: Modulation

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Topic: F.02. Animal Cognition and Behavior

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Title: Threat-elicited defensive behavior is modulated by designer receptor manipulation of locus coeruleus

Authors: *Y. GU[1], R. M. SEARS[1], J. E. LEDOUX[1,2]
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Abstract: Dysregulation of threat processing in the amygdala may contribute to the etiology of fear and anxiety disorders. Given that pharmacological treatments for fear and anxiety disorders often target monaminergic systems, it is possible that changes in tonic levels of monamine neuromodulators in the amygdala may contribute to these disorders. We are using Designer Receptors Activated by Designer Drugs (DREADDs) to increase or decrease endogenous noradrenergic tone in central amygdala (CeA) through intraperitoneal or intracranial clozapine-N-oxide (CNO) injections during discrete phases of a cued Pavlovian threat conditioning paradigm. Our findings support the hypothesis that tonic changes in norepinephrine in the amygdala profoundly affect the expression of both defense responses elicited by unconditioned and conditioned threats.

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Poster

468. Fear and Aversive Memories: Modulation

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Title: Revaluation of opposing reinforcers of avoidance behaviour

Authors: *A. B. FERNANDO[1], G. P. URCELAY[2,3], A. C. MAR[2,3], A. DICKINSON[2,3], T. W. ROBBINS[2,3]
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Abstract: Instrumental avoidance behaviour persists even in the absence of an aversive event. The sources of its reinforcement and the psychological processes that support its persistence have yet to be determined. Rats were trained on a free-operant lever- press avoidance paradigm to avoid shock and produce a contingent auditory safety signal. Using a novel revaluation procedure we selectively revalued these two sources of reinforcement; the negative footshock reinforcer or the positive safety signal reinforcer through presentations of either reinforcer with injections of analgesic agents. Following this revaluation procedure, behaviour was tested in extinction to assess the sensitivity of avoidance behaviour to a change in value of either reinforcer. The effectiveness of our revaluation procedures were confirmed with reinforced tests where the revalued reinforcer was presented during the session leading to changes in avoidance behaviour. We demonstrate that different associative processes support the different response-reinforcer contingencies present in avoidance behaviour. Furthermore, the revaluation procedures revealed specific neurobiological mechanisms for the revaluation of each reinforcer, suggesting different mechanisms for appetitive (safety signal) versus aversive (footshock) reinforcers of avoidance behaviour. These data therefore provide novel theoretical and clinically relevant findings in understanding the development of maladaptive avoidance behaviour.

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Poster

469. Cocaine

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Title: Chronic cocaine decreases GFAP of astrocytes in rat brain characterized by optical imaging

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Abstract: Astrocytes are involved in control of synaptic transmission, neuroplasticity and cerebral blood flow. Although studies have shown that stimulants drugs such as cocaine affect the function of astrocytes, it is still unclear how chronic cocaine exposure would affect astrocytes in the brain, especially in regions in the drug reward including the medial prefrontal cortex (mPFC), nucleus accumbens core (NAcc) and dorsal striatum (Dstr). The goal of this project is to image the morphological distribution of astrocytes in various brain regions, and to characterize its potential changes induced by chronic administration of cocaine. Adult male Sprague-Dawley rats (250-300g) were divided into two groups (chronic cocaine group and control group). Cocaine (cocaine HCl; 5mg/ml i.p.) was administrated once a day (35mg/kg, i.p.) for 14 days to the chronic cocaine group and 0.9% saline was administered to control rats. After a 24 hour withdrawal period, the animals were fixed using formalin perfusion. The brains were dissected and stored in sucrose solution for 1-2 days. Afterwards, they were embedded in OCT compound and frozen in -80^{o}C. For microscopic imaging, the brains were sectioned at -20^{o}C at 20μm on the Leica CM1850 cryostat. Glial fibrillary acidic protein (GFAP) was labeled to identify the astrocytes in the brain tissue through immunohistochemistry. Confocal microscopy was used to image the brain sections on the Zeiss LSM 510 at 20x magnification to determine the distribution of astrocytes in different brain regions. By using Image-J, the ratio of the pixel area of the GFAP fluorescence over that of the background intensity (RGFAP/Backgr) was calculated for each image to quantify its density in the different regions of the brain. We observed that the distribution of astrocytes differed between brain regions; while the astrocytes were distributed relative-uniformly in the cortex (e.g., mPFC and somatosensory cortex), in the NAcc the GFAP showed a decreasing anterior to posterior gradient. In addition, compared with the control rats, the GFAP signal was decreased significantly in the brain of chronic cocaine animals. Specifically, RGFAP/Backgr values in control versus cocaine groups corresponded to 10.3%$\pm$1.1 versus 4.4%$\pm$0.3 in mPFC, 8.2%$\pm$1.2 versus 2.3%$\pm$0.3 in Dstr, and 7.1%$\pm$3.7 versus 3.1%$\pm$0.4 in NAcc. These preliminary results indicate that chronic exposure of cocaine might affect astrocytes which might contribute to the changes in neuronal activity reported in the cocaine abusers.

Disclosures: S. Sundaresh: None. **K. Clare:** None. **N.D. Volkow:** None. **C. Du:** None. **Q. Zhang:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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T32 EY007125

Title: Effects of short-term cocaine self-administration on neural signals related to flexible decision-making in the rostral striatum

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Abstract: Drug-induced changes in frontostriatal circuits are believed to play a pivotal role in the transition from voluntary drug use to compulsive drug-associated behaviors in addiction. While much of the research in this area has focused on how addictive drugs enhance striatal-mediated habit learning processes, little has examined how drugs might disrupt striatal activity related to the control of flexible, goal-directed decision-making. The lack of research in this area is due, in part, to the fact that the role of the striatum in flexible decision-making in normal (i.e. drug naïve) circumstances is not well understood. Therefore, the goal of the current research was to determine 1) how the striatum is involved in normal (drug-naïve) flexible decision-making, and 2) how striatal signals related to flexible decision-making are altered following drug (cocaine) exposure. Neural activity was examined in two monkeys performing a novel monkey adaptation of the Wisconsin Card Sorting Test (WCST), a classic and well-studied paradigm for understanding goal-directed cognition. On each trial of our task, monkeys were presented with a series of three stimuli that differ in shape (circle, triangle, or star) and color (cyan, magenta, or yellow). Monkeys were then required to choose one of these stimuli based on a rule (a specific color or a shape) and maintain the rule for 10,15, or 20 trials. Correct choices were followed by positive visual feedback and a reward, while incorrect choices were followed by negative visual feedback and no reward. Rule changes were not cued and new rules had to be learned through trial and error. Following training on the WCST, we recorded firing rate activity in the rostral regions of the ventral striatum, caudate, and putamen while two monkeys performed this task. Our analyses were designed to detect switching signals (defined as a significant modulation of firing rate activity immediately prior to behavioral acquisition of a new rule). Our cocaine-naïve data suggest that neurons throughout the striatum are involved in signaling the need to switch behavior. Notably, we found that a number of neurons within the ventral striatum (which is typically thought to be involved only in simpler forms of flexible decision-making) also demonstrated switch signals similar to those found in the caudate and putamen. Our preliminary cocaine-exposed data suggest that, following cocaine exposure, striatal switch signals are reduced in the caudate nucleus. We anticipate that this work will lead to a greater understanding

of circuit level changes caused by addictive drugs, and ultimately towards the design of novel, rational treatments for drug addiction.

Disclosures: **B.J. Sleezer:** None. **B.Y. Hayden:** None. **T.C. Blanchard:** None.

Poster

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Topic: F.03. Motivation and Emotion

Title: Long-term consequences of the excessive self-administration of cocaine on brain vascular structure

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Abstract: Cocaine is known to produce vascular damage in cocaine abusers. Despite considerable clini-cal data, very few studies have investigated the neurovascular modifications induced by chronic cocaine intake in animal models. Here, we investigated whether excessive cocaine taking could produce structural changes in the brain vasculature and whether these modifi-cations persist after discontinuation of drug taking. For this, after 7 days of cocaine self-administration training (2h/day), rats were given either short-access (1h/day) or long-access (6h/day) to cocaine self-administration for 20 days. A third group did not undergo self-administration and served as control. At the end of self-administration, rats underwent forced abstinence and brain samples were obtained after 7 or 28 days for immunohistochemical staining of endothelial cells. The length of vessels was quantified in several regions including the nucleus accumbens (NAc) Core and Shell, the Ventral Tegmen-tal Area (VTA), the cingulate cortex (Cg) and several nucleui of amygdala using stereological methods. We found that long-access to cocaine produced significant decrease in the length of blood vessels in the Shell of the nucleus accumbens after 7 days of withdrawal which tended to return to basal levels after 28 days. Moreover, an increase in the length of blood vessels was observed in the Central Amygdala after 28 days of withdrawal. Exposure to co-caine did not significantly alter the length of vessels in the other regions. These results sug-gest that chronic long-access to cocaine induces selective modifications in vascular structure in regions involved in drug addiction, which could contribute to the pathophysiology of ad-diction.

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA025636

VA BLR&D 1IO1BX000782

Title: Longitudinal structural changes following long term cocaine self-administration in rhesus macaques: Relationship with motivation of drug administration

Authors: ***H. P. JEDEMA**[1], A. BONNER[2], J. N. PORTER[3], H. J. AIZENSTEIN[1], C. W. BRADBERRY[1,4]
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Abstract: Addiction is a complex neurobiological disorder affecting multiple brain regions. Clinical studies have indicated structural changes in human cocaine users compared to matched control subjects in frontal and temporal cortical regions, as well as the cerebellum. It is unknown whether these differences reflect structural differences induced by chronic (poly) drug use or pre-existing structural differences potentially related to enhanced vulnerability to addiction. Because of the similarity in brain structure and response to drugs of abuse in monkeys, we compared the longitudinal impact of chronic intravenous cocaine self-administration (up to 6 infusions 0.5mg/kg each, 4days/week for 12 months) in adult rhesus macaques (n=8) with control subjects (N=6) in previously defined volume of interest regions using atlas-based morphometry and MRI (Siemens Allegra 3T). In addition, we examined the correlation between the motivation to self-administer cocaine (average time to self-administer each infusion) and structure at baseline as well as structural changes observed following prolonged cocaine self-administration. We observed significant differences in relative structural changes in gray matter in frontal cortical (BA 45), temporal (amygdala), and cerebellar regions between cocaine self-administering and control subjects. In the cocaine group, we found a significant correlation between the relative size of orbital frontal cortex at baseline and the subsequent time to self-administer cocaine. In

addition, we observed significant correlations between structural changes and the time to self-administer cocaine in the ventral prefrontal cortex (BA 25), the pre-supplementary motor area, and the cerebellum. These data suggest chronic cocaine self-administration may lead to structural changes in lateral prefrontal, temporal, and cerebellar regions. In addition, pre-existing differences in orbital frontal cortex may result in greater vulnerability to cocaine self-administration in monkeys. These observations provide further support for non-human primate models of cocaine addiction and underscore the importance of frontal cortical and cerebellar brain regions in addiction.

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Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant DA015369

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Title: Rapid transient plasticity in both dopamine D1 and D2 receptor expressing medium spiny neurons in accumbens mediate relapse to cocaine seeking

Authors: ***J. A. HEINSBROEK**, W. GRIFFIN III, A. C. W. SMITH, L. N. LUDERMAN, M. D. SCOFIELD, P. W. KALIVAS, C. D. GIPSON
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Abstract: Medium spiny neurons (MSN) in the nucleus accumbens comprise a critical component in addiction circuitry that links internal motivational states and environmental stimuli to motor action within basal ganglia. During relapse to cocaine seeking, a rapid, transient LTP-like effect is observed in these cells that is characterized by increased spine head diameter (d_h) and AMPA/NMDA ratio. Furthermore, increased activity of matrix metalloproteinase 9 (MMP-9) in the nucleus accumbens contributes to this transient potentiation through signaling within the extracellular matrix surrounding these MSNs. Classically, D1 expressing MSNs are thought

to mediate increased motivation via projections to the ventral mesencephalon. Conversely, D2 expressing MSNs projecting through the ventral pallidum to subthalamic nucleus and mediodorsal thalamus are thought to mediate decreased motivation. Recent data from our lab suggests, however, that both D1 and D2 expressing neurons project to the VP and that the projection from nucleus accumbens to ventral pallidum, but not to the ventral mesencephalon is critical for reinstatement of cocaine seeking behavior (Stefanik *et al.*, 2013; Kupchik *et al.* this conference). To establish whether relapse-mediated synaptic changes in MSNs are specific to D1 or D2 expressing neurons, D1-eGFP reporter mice were trained to self-administer cocaine. After acquiring stable responding, animals underwent extinction training and were either sacrificed before (T=0) or 15 min following presentation of contingent cocaine-associated cues (T=15) for immunohistochemistry and morphological analysis (Gipson *et al.*, 2013). In both D1 positive MSNs (D1(+)) and D1 negative MSNs (putative D2; (D1-)), potentiation of d_h was observed at T=0, compared to saline controls. At T=15, further potentiation of d_h occurred specifically in D1(+) MSNs. These data suggest that synapses on both D1 and D2 expressing MSNs are potentiated after cocaine self-administration but that D1(+), and not D1(-) expressing MSNs may be necessary for cue-induced reinstatement. Ongoing experiments are aimed at validating the abovementioned results using D2-mCherry reporter mice, the activity of MMP-9 around D1 and D2 MSNs and dissection of D1 and D2 basal ganglia circuitry.

Disclosures: **J.A. Heinsbroek:** None. **W. Griffin III:** None. **A.C.W. Smith:** None. **L.N. Luderman:** None. **M.D. Scofield:** None. **P.W. Kalivas:** None. **C.D. Gipson:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant PO1 DA031656

Title: Rapid cocaine induced sensitization of phasic dopamine transmission in the nucleus accumbens shell

Authors: *M. A. BRYAN, B. F. SINGER, B. J. ARAGONA, T. E. ROBINSON
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Abstract: Repeated exposure to psychostimulants, such as cocaine and amphetamine, produces a progressive increase in their psychomotor effects, a phenomenon known as behavioral sensitization. The expression of sensitization has been associated with increased dopamine (DA) signaling within the nucleus accumbens (NAc). A wealth of evidence suggests DA transmission within the NAc mediates the rewarding and motivational effects of addictive drugs, underlying drug craving and thus subsequent drug seeking and relapse. While relatively little is known about the mechanism mediating enhanced DA release from these dopaminergic terminals, recent studies have indicated that initial cocaine exposure enhances the excitability of DA neurons in the VTA within three hours, leading to the induction of long-term potentiation (LTP). We hypothesized that these events may subsequently result in sensitization of DA release in the NAc shell. Given the LTP changes occur within a 3 hour time period, this study investigated whether changes in evoked DA release in the NAc would occur on a similar time scale. Fast scan cyclic voltammetry (FSCV), a highly temporally and spatially resolute method, was used to detect DA transmission within the NAc shell following first cocaine exposure and second cocaine exposure 2 hours later. Sensitized DA release was exhibited during the first 5 minutes following the second cocaine injection, as indicated by an increase in the average amplitude of DA transients, suggesting the induction of sensitization and LTP share a common time course. This evidence supports a model in which induction of cocaine-evoked DA sensitization in the NAc is dependent on an LTP-like mechanism at the VTA, shedding further light on the neurobiology of drug addiction, craving and relapse.

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Poster

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Title: Psychosocial stress-induced reinstatement of cocaine-seeking behavior in rats

Authors: *D. F. MANVICH[1], T. A. STOWE[2], D. WEINSHENKER[1]
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Abstract: A prominent feature of cocaine abuse disorders is the high frequency of relapse events that occur even following prolonged periods of abstinence. Stress-induced relapse is frequently modeled in experimental animals using the reinstatement procedure, but the overwhelming majority of these studies employ physical (e.g. footshock) or pharmacological (e.g. yohimbine) stressors which lack face and translational validity. Social defeat stress has been proposed as an ethologically-valid psychosocial stressor in rodents and has been argued to closely model the forms of psychosocial stress experienced by drug abusers that promote craving and relapse. The goal of this study was to develop and characterize a novel preclinical model of stress-induced relapse to cocaine use in rats using social defeat stress. Adult male Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a continuous schedule of reinforcement in daily 2 hr sessions for 20 days. On days 11, 14, 17, and 20, subjects were removed from the chamber immediately following the session and either subjected to social defeat stress using a conventional resident-intruder procedure or placed into an empty cage. Discrete environmental stimuli (odorous and tactile) present within the operant chamber on these days signaled the impending social defeat stress or empty cage exposure. Each social defeat episode was video-recorded and the activity of the intruder was scored later by a trained observer. Extinction sessions began on day 21, during which presses on the formerly-active lever no longer resulted in cocaine infusions. Once extinction criteria were satisfied, the rat was placed in the operant chamber along with the discrete cues that previously signaled impending social defeat stress or empty cage exposure and allowed to lever-press under extinction conditions. Results indicated that, compared to their non-stressed counterparts, animals reexposed to psychosocial stress-predictive cues exhibited significant reinstatement of cocaine-seeking behavior. Ethogram analyses of social defeat encounters revealed that the average amount of time the subject engaged in “passive” behaviors (e.g. freezing, submissive supine posture) was strongly and positively correlated with subsequent reinstatement magnitude. These studies are the first to describe a model for psychosocial stress-induced relapse of cocaine use in rodents and suggest that distinct coping behaviors may be used to predict individual propensity to exhibit drug-seeking behavior in response to perceived impending psychosocial stress.

Disclosures: D.F. Manvich: None. **T.A. Stowe:** None. **D. Weinshenker:** None.

Poster

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Title: Cue-induced cocaine seeking involves nucleus accumbens core glutamate overflow mediated by mGluR2/3 and mGluR5

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Abstract: Addiction to cocaine produces long-lasting, stable changes in brain synaptic physiology that might contribute to the vulnerability to relapse. Cues associated with cocaine use can precipitate relapse, and recently we showed that cue-induced cocaine relapse is associated with rapid, transient synaptic plasticity within the nucleus accumbens core (NAcore). It was unknown, however, if cues associated with cocaine induce glutamate overflow within the NAcore as shown with administration of cocaine itself and cue-induced heroin and nicotine seeking. When cocaine seeking was reinstated by presentation of contingent conditioned cues, there were parallel increases in behavioral responding and NAcore extracellular glutamate. Reverse dialysis of the mGluR2/3 agonist LY379268 into the NAcore inhibited both glutamate overflow and behavioral responding, indicating that pre-synaptic mGluR2/3 receptors mediate cocaine cue-induced glutamate overflow in the NAcore. In contrast, when the mGluR5 antagonist MTEP was reverse dialyzed, NAcore glutamate overflow remained intact yet behavioral responding was inhibited. These results confirm that cocaine cue-induced glutamate overflow activates post-synaptic mGluR5 receptors and promotes cue-induced reinstatement of cocaine seeking. Moreover, glutamate overflow onto these receptors may mediate rapid, cocaine cue-evoked synaptic potentiation in the NAcore. We will next determine how the mGluR2/3 agonist and mGluR5 antagonist affect the transient synaptic potentiation produced by synaptic glutamate overflow.

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Poster

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Title: Transient synaptic plasticity induced by relapse to a cocaine cue is reversed by giving access to cocaine

Authors: *S. M. SPENCER, P. W. KALIVAS
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Abstract: Chronic cocaine produces long-lasting changes in synaptic strength in the nucleus accumbens (NAc) that can be quantified as alterations in dendritic spine structural plasticity. Specifically, chronic cocaine increases dendritic spine density and spine head diameter of NAc core medium spiny neurons (MSNs). These enduring alterations in NAc synaptic plasticity are believed to contribute to compulsive drug seeking in animal models and "relapse" in humans after a period of withdrawal. Recently, we have shown that reinstated drug seeking elicited by cocaine-conditioned cues, cocaine-paired context, or non-contingent administration of the drug itself leads to a further rapid, transient augmentation in dendritic spine plasticity. We postulated that the transient synaptic potentiation induced by a cue is mediating motivation to seek drug and that access to cocaine would suppress both the synaptic potentiation and drug seeking. To evaluate this hypothesis, we modified our reinstatement procedure to restore drug use after a 10 min period of drug seeking induced by drug-associated cues without drug. In this way, cocaine-induced reinstatement more closely resembles a cocaine relapse event following exposure to cocaine cues as would occur in the human condition. We find that gaining access to cocaine reversed the cue-induced increase in spine diameter and density, while animals accessing only saline showed transient plasticity. We next hypothesized that the restoration of cocaine reduces motivation and synaptic potentiation by promoting dopamine release in the NAc and producing drug-induced satiety. In line with this hypothesis, spine head diameter rebounds in cocaine-restored animals when they progress to a within session extinction. Additional studies are ongoing to explore the specific mechanism by which dopamine may inhibit synaptic potentiation.

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Poster

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Title: Long-access cocaine self-administration induces persistent drug/cue associations revealed by cocaine-conditioned 50 kHz USVs but not conditioned overt behaviors

Authors: *J. M. RENO, JR[1], E. M. KUSEY[2], T. SCHALLERT[3], C. L. DUVAUCHELLE[2]
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Abstract: A loss of control in drug taking, increased drug seeking, and negative life consequences that stem from use are hallmarks of drug abuse that distinguish it from recreational drug use. Emotional precedents play an important part early in the relapse-abstinence cycle. In the current study, ultrasonic vocalizations (USVs) were used as an index of emotional response to cocaine experience in adult Sprague-Dawley rats. USVs, locomotor activity and lever responses were assessed during long-access (LA, 6 hrs) cocaine self-administration sessions (0.75mg/kg/infusion). Similar to our previous findings during shorter (1hr) cocaine self-administration sessions, LA rodents emit a greater number of 50 kHz USVs during pre-drug intervals in the drug-taking environment (operant chamber) compared to saline controls. However, unlike animals exposed to cocaine during short sessions, during extinction sessions, conditioned locomotion and lever responding exhibited by LA rodents extinguish prior cocaine-conditioned 50 kHz USVs. Indeed, cocaine-conditioned 50 kHz USVs, which occur both before and after lever availability, continue to be elicited by LA rats well into the extinction phase. These findings indicate that long-access cocaine self-administration sessions induce persistent drug-cue associations that are revealed by cocaine-conditioned USVs, but not by overt behaviors such as conditioned locomotion and drug-seeking lever responses. By reflecting social and/or emotional reactions to previously encountered drug/cue associations, cocaine-conditioned USVs are sensitive targets for pharmacological and therapeutic interventions and uniquely suited for exploring compounds acting on emotional predecessors to drug relapse behaviors.

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Poster

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HN Mai, TTL Nguyen, THT Tu, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea

Title: Role of glutathione peroxidase1 gene in the oxidative burdens mediated by cocaine drug dependence in the striatum and liver of mice

Authors: ***E.-J. SHIN**, H.-N. MAI, T.-T. L. NGUYEN, T.-H. T. TU, Y. NAM, H.-C. KIM
Neuropsychopharmacol. and Toxicol. Program, Col. of Pharm., Kangwon Natl. Univ., Chunchon, Korea, Republic of

Abstract: Accumulating evidences have suggested that oxidative stress and its associated impaired antioxidant system are implicated in cocaine-induced intoxication. We investigated here whether psychotoxicity and hepatic changes induced by cocaine induce oxidative stress. We examined parameters of the oxidative stress (MDA, ROS, protein carbonyl) and enzymatic antioxidant [superoxide dismutases (SOD), glutathione peroxidase (GPx), catalase, glutathione-S-tranferase (GST)] in the striatum and liver after the induction of cocaine psychotoxicity. Cocaine-induced conditioned place preference parallels behavioral sensitization. Importantly, this psychotoxicity-mediated oxidative burdens are in line with hepatic oxidative burdens as reflected by the increase in oxidative stress (MDA, ROS and protein carbonyl) and the decrease in the activities of antioxidant enzymes (GPx, GST, SOD, catalase). As GPx activity is the most susceptible to cocaine intoxication, we next applied GPx1 gene knockout (KO) and GPx1 gene transgenic mice (GPx1-Tg). These oxidative stresses were more pronounced in GPx1 KO than in wild-type mice, while they were less pronounced in GPx1-Tg than in non-Tg. Combined, our results reflect that cocaine-induced psychotoxicity modulates hepatic oxidative burdens and that potential role of GPx1 gene in blocking cocaine-induced intoxication, although precise

interactive signaling between cerebro- and hepatic-system remains to be further examined [HN Mai, TTL Nguyen, THT Tu, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea. This research was supported by a grant (14182MFDS979) from Ministry of Food and Drug Safety in 2014, Republic of Korea].

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Poster

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Title: Chronic cocaine is associated with reduced cerebral blood flow and with increase of microvascular density in the rat brain

Authors: ***Q. ZHANG**[1,2], J. YOU[1], P. LIU[1], W. WANG[2], Y. PAN[1], N. D. VOLKOW[3], C. DU[1]
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Abstract: Cocaine abuse increases the risk of life-threatening neurologic complications, e.g., strokes, hemorrhages, and transient ischemic attacks. About 25% to 60% of cocaine-induced strokes can be attributed to cerebral vasospasm and ischemia. Brain imaging studies have documented remarkable decreases in cerebral blood flow (CBF) and blood volume (CBV) in cocaine abusers. However, the mechanisms underlying cocaine-induced CBF reduction, cerebral vasospasm and ischemia are poorly understood. Here we use Doppler optical coherence tomography (DOCT) to quantitatively image cerebral blood flow (CBF) networks in the cortex

of rats with or without chronic cocaine exposure. By combining with ex-vivo fluorescence approaches, we assessed the effects of chronic cocaine exposure in microvasculature density in different brain regions. Adult male Sprague-Dawley rats (250-300g) were divided into two groups (chronic cocaine group and control group). Cocaine (cocaine HCl; 5mg/ml i.p.) was administrated once a day (35mg/kg, i.p.) for 14 days to the chronic cocaine group and 0.9% saline was administered to control rats. For in-vivo experiment, we used DOCT, which provides three-dimensional imaging of CBF cortical networks and quantification of CBF velocity from all of the vessels in the region. For the ex-vivo experiment, we intracardiacally infused FITC-Dextran (average mol wt 2,000,000 ; 50mg/ml in 0.9% saline. Following brain sectioning, microscopic fluorescence imaging was used to quantify the microvasucular density in various brain regions, including middle prefrontal cortex (mPFC), somatosensory cortex (Ssc), dorsal striatum (DStr), nucleus accumbens (NAc) and hippocampus (Hpc). We show that CBF was reduced by 30-50% in the brain of cocaine exposed rats compared to controls whereas the microvascular density was increased in several brain regions , including mPFC (318.6±13.9 vs 239.3±8.7), Ssc (362.3±6.9 vs 273.7±4.0) , DStr (221.0±5.1 vs 155.7±26.6)and NAc (268.9±9.5 vs 180.5±20.4) but not in hippocampus (194.5±9.7 vs 198.0±19.2). These results suggest that reduction in CBF trigger angiogenesis to compensate for cocaine induced vasoconstriction. However the fact that CBF was reduced despite increase in the numbers of vessels suggests that angiogenesis might be insufficient to compensate for cocaine's effects.

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Poster

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Program#/Poster#: 469.13/VV35

Topic: F.03. Motivation and Emotion

Title: Self-administration and cardiovascular assessments of a cocaine-amphetamine combination drug in the adult male rat

Authors: *J. D. TOOT, T. PRINGLE, M. BENNETT, M. HACKMAN, K. LANDIS, P. ATTERSON
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Abstract: The objectives of this study were to: 1) assess the self-administration testing paradigm utilizing multiple drugs under a Latin Square design, 2) demonstrate the positive reinforcing effects in this model with the combination test drug cocaine-amphetamine, 3) monitor cardiovascular and general activity endpoints via radiotelemetry, 4) demonstrate the ability of cocaine-amphetamine to be self-administered in the rat within the scope of the regulatory guidelines. The animals used for this study consisted of adult Sprague Dawley male rats, at approximately 16 weeks of age at the initiation of training. Rats were trained to press the active lever in order to receive an IV infusion (reinforcer) of the training/reference compound, cocaine, during a maximum 1 hour test session on a fixed ratio schedule. This was followed by alternating between extinguishing that behavior with saline and reinstatement/substitution sessions with cocaine, amphetamine, or cocaine/amphetamine (0.32, 0.05, or 0.32/0.05 mg/kg/infusion, respectively). Using this paradigm, animal responses were measured as the average number of infusions/session. Animals were also monitored during testing for cardiovascular related parameters (systolic blood pressure, mean arterial pressure, and heart rate) and general activity via a radiotelemetry transmitter implanted into the femoral artery. During testing, the number of cocaine, amphetamine, and cocaine-amphetamine infusions were increased compared to the infusions earned during the saline extinction sessions. However, the combination drug of cocaine-amphetamine resulted in a lower number of infusions as compared to the cocaine and amphetamine alone. As expected, cardiovascular measurements were elevated during the self-administration substitution sessions, as compared to the saline extinction sessions. The use of the self-administration testing paradigm, as well as the successful reference drug training and substitution level selection were able to adequately establish the positive reinforcing potential of cocaine, amphetamine, and cocaine-amphetamine. Therefore, these results further support the self-administration methodologies, particularly when used in conjunction with the radiotelemetry system monitoring key cardiovascular endpoints.

Disclosures: **J.D. Toot:** A. Employment/Salary (full or part-time):; WIL Research. **T. Pringle:** A. Employment/Salary (full or part-time):; WIL Research. **M. Bennett:** A. Employment/Salary (full or part-time):; WIL Research. **M. Hackman:** A. Employment/Salary (full or part-time):; WIL Research. **K. Landis:** A. Employment/Salary (full or part-time):; WIL Research. **P. Atterson:** A. Employment/Salary (full or part-time):; WIL Research.

Poster

469. Cocaine

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 469.14/VV36

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01-DA-032789

Title: The medial preoptic area modulates cocaine-induced locomotion in male rats

Authors: *R. G. WILL, J. R. MARTZ, T. HATTORI, J. M. DOMINGUEZ
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Abstract: Studies show that cocaine increases locomotion in all studied species. Recently published data points to the medial preoptic area (mPOA) of the hypothalamus as a modulator of cocaine-induced neural and behavioral response in rats. Whether the mPOA plays a similar role in cocaine-induced locomotion is not clear. To answer this question, male rats with neurotoxic lesions or sham lesions of their mPOA were given cocaine or saline and then placed in an automated open field chamber to measure and record motor activity. Cocaine-induced locomotor sensitization was also measured over a 4-day period after the initial exposure to cocaine. Results show that lesions of the mPOA enhanced cocaine-induced locomotion, while subjects with lesions exhibited higher cocaine induced Fos expression in the ventral palladium. These results support the modulatory role for the mPOA in cocaine-induced activity, which may occur via interactions with the ventral palladium.

Disclosures: R.G. Will: None. **J.R. Martz:** None. **T. hattori:** None. **J.M. Dominguez:** None.

Poster

469. Cocaine

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Title: Compulsive cocaine self-administration decreases cerebral blood flow and tissue oxygenation in the prefrontal cortex as measured by in-vivo optical neuroimaging

Authors: *C. DU[1], Y. PAN[1], S. WEE[2], K. PARK[1], N. ZHOU[1], J. YOU[1], G. KOOB[3], N. D. VOLKOW[4]
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Abstract: Deficits in prefrontal function and consequential loss of inhibitory control of pyramidal glutamatergic output neurons are hypothesized to play a crucial role in promoting compulsive cocaine use. Dysfunction of the prefrontal cortex may result both from the fact that cocaine directly affects cerebral blood vessels and neurons in the brain. While imaging technologies such as fMRI and PET have assessed the acute and chronic effects of cocaine on neuronal function, limitations of current techniques to differentiate vascular from neuronal effects at sufficiently high temporal and spatial resolution have prevented analysis of specific vascular and tissue metabolic changes. Here, we present a novel multimodel imaging platform developed in our laboratories that can distinguish the neuronal from the cerebrovascular effects of cocaine. We applied this approach to study functional changes in the prefrontal cortex of the rodent brain that follow compulsive cocaine self-administration. Our optical imaging platform (OFI) enables simultaneous image hemodynamics (including cerebral blood flow (CBF) and blood volume (CBV) and hemoglobin oxygenation and deoxygenation (StO2)) and intracellular calcium ([Ca2+]i) florescence (marker of neuronal activity), which allows us to monitor the neurovasculature, tissue metabolism and neuronal activity at high spatiotemporal resolutions over a large field of view. We use an animal model of compulsive intravenous (iv) cocaine self-administration that mimics human addiction. Naïve, short-access (ShA) and long-access (LgA) self-administering rats are then subjected to an iv cocaine challenge during imaging, and we concurrently measure the cerebrovascular responses (CBF, CBV), tissue oxygenation (StO2) and neuronal [Ca2+]i changes in the prefrontal cortex in response to cocaine. Our results showed that, a). Acute cocaine decreases CBF while increasing deoxyhemoglobin and increasing intracellular Ca content and that there is sensitization to these effects with LgA cocaine exposure; b). These sensitized responses with LgA chronic cocaine exposures increase the vulnerability of neuronal tissue to induce micro-ischemia and the consequent damage to cortical tissue from hypoxia. These results with a new imaging tool permits one to distinguish the vascular versus the neuronal responses of the brain in response to a pharmacological challenge, thus complimenting other neuroimaging modalities (e.g., PET, fMRI) for investigating brain functional changes such as those induced by cocaine abuse.

Disclosures: **C. Du:** None. **Y. Pan:** None. **S. Wee:** None. **K. Park:** None. **N. Zhou:** None. **J. You:** None. **G. Koob:** None. **N.D. Volkow:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Program#/Poster#: 470.01/VV38

Topic: F.03. Motivation and Emotion

Support: KAKENHI(17022052, 22300138)

Title: Single neuronal activity in the monkey orbitofrontal cortex related to reward value processing during decision-making

Authors: *T. SETOGAWA[1], T. MIZUHIKI[1,2], F. AKIZAWA[2], R. KUBOKI[2], N. MATSUMOTO[3], M. SHIDARA[1,2]
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Abstract: In our daily life, we often choose one item or action from several alternatives by considering their values and efforts to obtain them. To know the mechanism of such decision-making process, we developed a decision-making schedule task to obtain a reward and recorded single neuronal activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain areas for reward-guided behaviors. Two monkeys were initially trained to perform a reward schedule task. In this task, the monkey had to complete the schedule composed of 1, 2 or 4 trials of visual discriminations to earn 1, 2 or 4 drops of liquid reward. After the monkey learned this task, the decision-making schedule task was introduced. The decision-making schedule task was consisted of the decision-making part and the reward schedule part. In the decision-making part, two kinds of choice target were presented sequentially at the center of the computer monitor (these targets were called the first and second target, respectively). Brightness and length of the choice target were proportional to the amount of liquid reward (1, 2 or 4 drops) and the required number of the visual-discrimination trials (1, 2 or 4 trials) to be performed, respectively. After choice targets were presented sequentially, these two choice targets simultaneously reappeared on both sides of the fixation point in random order. Then the monkey was required to choose one of the two choice targets by touching the corresponding bar in the chair. Then, the chosen reward schedule task was started. We recorded from 191 neurons in the monkey OFC during the decision-making schedule task (137 and 54 neurons from each monkey). The choice target values were estimated by a temporal discounting model using the monkey's choice behavior, and then the relation between neuronal firing and the choice target values was analyzed. 20/191 (10.5%) of the recorded neurons showed a correlation

between current target values and firing rate in the first and/or second target presented period. In the second target period, the activities of 11/191 neurons were linearly related with difference in value of the two choice targets (11/191: 5.8%). 14/191 (7.3%) neurons showed larger/smaller responses when the two choice target values were close. Preliminary data suggest the neurons show different activity when the higher value option was chosen than when the lower one was chosen. These results suggest that OFC neurons play an important role in the decision-making by reward value information processing.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Support: Vidi grant from the Netherlands Organization for Scientific Research (NWO) (grant 452-11-015)

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Title: Early and late reorganization of amygdala connections following unilateral and bilateral destruction of the visual cortex

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Abstract: Several patients with destruction of the visual cortex retain the ability to discriminate and respond to the emotional value of stimuli presented in their clinically blind field (affective blindsight). This preserved ability involves the amygdala, as previously shown in fMRI studies. However, it is unknown whether destruction of the visual cortex induces plastic changes in amygdala cortical connections. Moreover, although it is well-known that post-lesion neural plasticity is influenced by the onset age of the lesion and by whether the lesion is unilateral or is

bilateral, the combined influence of these factors in amygdala connectivity has never been investigated. Here we used Diffusion Weighted MRI to investigate amygdala connectivity in two patients with cortical blindness and in ten age-matched controls. Patient GY has a unilateral destruction of his left V1 and an early lesion onset (at age 7), whereas patient TN has a bilateral lesion to the visual cortices and late onset (at age 52). Compared to controls, amygdala connections with occipito-parietal area and with premotor areas were significantly reduced in the damaged hemisphere of GY and bilaterally in TN. Conversely, connections with the ventral anterior temporal regions were significantly increased in the damaged hemisphere of GY and in both hemispheres of TN. Lastly, in patient GY we found a significant increase of inter-hemispheric connectivity. The present findings thus show that neuroanatomical reorganization in amygdala connections can occur after early as well as late onset of lesions to the visual cortex, and that a unilateral lesion yields interhemispheric compensatory processes.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Program#/Poster#: 470.03/VV40

Topic: F.03. Motivation and Emotion

Support: NIDA grant R01DA19028

NIMH grant R01MH097990

Title: Prefrontal mechanisms of hierarchical reinforcement learning

Authors: *F.-K. CHIANG[1], J. D. WALLIS[1,2]
[1]Psychology, [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA

Abstract: Computational models of reinforcement learning (RL) are the leading theoretical framework for understanding how humans and animals take actions to maximize rewards in uncertain environments. Computational RL, especially in the temporal-difference learning model, is accomplished by computing positive or negative reward prediction errors (RPE). Neurons encoding RPE have been seen in several brain areas, including the anterior cingulate cortex (ACC), as well as dopamine neurons in the midbrain. Recent theoretical studies have proposed modifications to the conventional RL models in order to allow them to accommodate

more complex hierarchical behavioral structure that is typical of the real word. In particular, hierarchical reinforcement learning (HRL) models have been developed that incorporate two key innovations. First, attaining a goal is broken down into component subgoals, while the individual actions necessary to attain the subgoal are chunked together. Second, attainment of the subgoal is capable of generating a prediction error signal. These signals are referred to as 'pseudo-rewards', since they are not primary rewards, but they can nevertheless reinforce behavior. The neural architecture that could be responsible for implementing HRL remains unclear. To determine whether there is evidence for these two innovations of HRL at the single-neuron level, we devised a task with static and dynamic configurations for use in monkeys that requires the accomplishment of a subgoal before a goal can be attained. Furthermore, the steps to acquire subgoal can be changed dynamically allowing us to generate pseudo-reward prediction errors. During performance of this task, we recorded 368 neurons in dorsolateral prefrontal (DLPFC, 132), orbitofrontal (OFC, 130) and ACC (106) simultaneously in one subject to examine whether they encode RPE or pseudo-reward prediction errors (PPEs). Overall, 24%, 17%, 42% of neurons in DLPFC, OFC, and ACC encoded the parameters of the original configurations, and 17%, 15%, 22% of neurons in DLPFC, OFC, and ACC responded to changes of the configurations. The most common response to a change in the configuration was an RPE and there was little evidence for encoding of PPEs. We discuss the implication of these results for models of hierarchical behavior.

Disclosures: F. Chiang: None. **J.D. Wallis:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: NSF Grant SES-0820316

NIH Grant NIAAA R01AA018736 (CRCNS)

Title: Single neuron coding of fairness in the human anterior midcingulate cortex

Authors: *D. M. DEVILBISS, R. L. JENISON
Univ. of Wisconsin, Madison, WI

Abstract: The anterior midcingulate cortex (aMCC) has been shown to encode the negative perception provoked by unfairness, and more generally the anticipation of intense aversive stimuli. However, the neural coding of fairness and punishment-related decision-making in the aMCC remains poorly understood. The Ultimatum Game is a widely used bargaining paradigm for economic decision-making, and has been used to reveal the influence of unfairness on choice. Typically, one player (the proposer) is endowed with a sum of money and offers a portion to the second player (the responder). If the responder accepts the offer, both participants keep the suggested amounts otherwise each player earn nothing. When proposals fall below 20% of the endowment, the responder rejects it approximately half of the time. The current study examined aMCC neuron spiking activity recorded from patients undergoing diagnosis and, later, surgical treatment for pharmacologically intractable epilepsy. Anatomical location of the recording electrode microcontacts within the aMCC were confirmed with high resolution MRI. Subjects acted as responders in a series of 80 trials of the Ultimatum Game over two blocks. Endowments ranged from $2 to $25 and proposals ranged from 0 to 50% of the endowment. For example, in each trial the participant first saw a picture and the name of a person making an Ultimatum offer. Next, the participant was presented with the offer (e.g., “Sarah gets $10, you get $2”) and allowed an unlimited time to consider the offer and respond (“Accept” or “Reject”) with a button press. We investigated whether single and multi-unit activity covaried with the responder’s acceptance or rejection of the proposer’s offers. We modeled the neural spiking activity using non-parametric Peri-Event Time Histograms and a generalized linear model (Poisson-GLM) that models the moment-by-moment correlation with the decision to accept or reject the proposer’s offer. Our results demonstrate that aMCC neurons can code multiple aspects of the Ultimatum Game including the perceived fairness in the proposed division of money.

Disclosures: **D.M. Devilbiss:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NexStep Biomarkers, LLC., Cerora, Inc.. **R.L. Jenison:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: Independent Starting Grant (284366; Emotional Learning in Social Interaction project) A Olsson

Title: Observational extinction attenuates learned fear by recruitment of the vmPFC in humans

Authors: *J. HAAKER[1,2], A. GOLKAR[2], I. SELBING[2], A. OLSSON[2]
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Abstract: Observational extinction attenuates learned fear by recruitment of the vmPFC in humans Much of what humans and other animals learn about the environment comes through social forms of learning, such as observing the experiences of other individuals (so called *demonstrators*). Previous research has shown that observing a demonstrator's experience of safety to a feared stimulus (*observational* or *vicarious* fear extinction) leads to superior recall of extinction memory as compared to direct extinction by blocking the return of the conditioned fear response (Golkar et al 2013). Critically, this effect only emerged when the demonstrator was safe, and not when the demonstrator was aversively reinforced (i.e. observational reinforcement). To examine the neural correlates underlying the efficacy of this form of social safety learning, we contrasted the hemodynamic responses to directly extinguished stimuli after observational extinction versus observational reinforcement learning. We found that, compared to observational reinforcement, observational extinction resulted in superior recall of extinction memory that was accompanied by increased activity in the ventromedial prefrontal cortex (vmPFC). In addition, reinstatement to the observational reinforced cue was mediated by responses in the amygdala. Our findings suggest that observational extinction relies on similar neural mechanisms as direct extinction together with social cognitive processes that enhances the strength of the safety learning.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: NIDA R01DA033369

NIDA R01DA031579

NIH P30 DA023026

Title: Individual differences in proactive and reactive threat responses during signaled shock avoidance in humans

Authors: *A. X. GORKA, K. S. LABAR, A. R. HARIRI
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Abstract: Avoidance behaviors are frequently observed in certain anxiety disorders including PTSD. However, active avoidance is infrequently assessed in laboratory settings. Research in rodents has suggested that an animal's tendency to reactively respond with freezing behaviors is inversely correlated with its tendency to respond proactively with active avoidance, and that these responses are mediated through different sub-nuclei of the amygdala. However, these patterns have only been investigated in rodents with a limited range of threat responses. We set out to assess the relationship between proactive and reactive responses in humans during an auditory signaled shock avoidance task. Participants lifted their index finger in response to auditory stimuli. Skin conductance responses, EMG signal, reaction times, and accuracy were compared between shock trials, during which the delivery of an electrical shock was contingent on performance, and control trials. Increased skin conductance and EMG responses were observed during shock trials in addition to faster reaction times and improved performance (all p's $< .05$). Individual differences in threat responses were subjected to a Principal Component Analysis. Accurate responses during shock trials loaded negatively (-.443) onto the first principal component while skin conductance responses loaded positively (.780). These results suggest that proactive and reactive responses can act in opposition to each other across a wide range of responses, and that this interplay can be observed in human participants. Proactive responses are mediated by distinct neural circuits, and active avoidance may be helpful in assessing the symptomatology of PTSD and other mood and anxiety disorders.

Disclosures: **A.X. Gorka:** None. **A.R. Hariri:** None. **K.S. LaBar:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: Michael J. Fox Foundation

NIH RO1MH076136

Title: Machine learning of whole-brain connectivity patterns predicts dopaminergic state

Authors: *R. T. GERRATY[1], L. SCHMIDT[1], N. ROUHANI[1], E. K. BRAUN[1], T. D. WAGER[2], I. KAHN[3], D. SHOHAMY[1]
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Abstract: Virtually all aspects of complex behavior involve the orchestration of widespread networks throughout the brain. Neuromodulators such as dopamine are likely to play an important role in modifying brain-wide circuits, both in health and in disease. Yet, the relationship between network-level connectivity and specific neurotransmitters remains poorly understood. In this study, we sought to characterize the relationship between dopamine and large-scale functional connectivity in patients with Parkinson's disease, which involves severe dopamine depletion resulting in pronounced motor deficits as well as affective and cognitive impairments. Neurobiological and computational models suggest that dopamine is crucial for shaping expectations about reward and that expectation alone may influence dopamine levels. Here we separated the pharmacological and psychological determinants of dopamine levels in the brain by using a placebo dopaminergic manipulation in patients with Parkinson's disease. In the early to moderate stages of the disease, dopamine loss is most pronounced in nigro-striatal pathways, providing an opportunity to address questions about whether dopamine loss in a relatively selective circuit relates to system-wide changes in connectivity. We used a within-subject design to test patients under three conditions: OFF drug, ON drug (L-Dopa, a dopamine precursor), and placebo. Large-scale measures of functional connectivity were used to provide a characterization of distributed brain function. These patterns were extracted from fMRI data acquired during a partial reinforcement task and used to train sparse multinomial logistic regressions (SMLR) to predict dopaminergic state. This multivariate measure of distributed connectivity resulted in significantly accurate prediction of drug state across ON and OFF conditions, suggesting that neurotransmitter levels effect widespread changes in functional connectivity. Weights from SMLR training were used to characterize the contribution of networks of regions to discriminability of drug state, suggesting specific effects of dopamine on connectivity patterns. These results both further our understanding of the relationship between expectancy, neurotransmitter function, and large-scale brain organization, and provide insight into a possible mechanism for the development of widespread deficits in Parkinson's disease.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Program#/Poster#: 470.08/VV45

Topic: F.03. Motivation and Emotion

Support: CIHR Grant

Title: Predicting pain from aversive fear conditioning modelling

Authors: *V. TAYLOR[1,3,4], M. ROY[5,6], M. LARAMÉE[2], P. RAINVILLE[2,1,3,4]
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Abstract: Animal studies show that a fear conditioned stimulus (CS+) induces analgesia mediated by descending inhibitory signals to the spinal cord from the amygdala. Amygdala and brainstem function also reflects encoding of prediction errors (differences between expected and actual outcomes) during classical fear conditioning. However, little is known of the relationship between learning indices derived from classical conditioning theories and spinal, as well as supra-spinal pain responses to a nociceptive US. The interaction between conscious awareness of CS-US pairing and the relationship between learning and pain responses also remains unknown. This psychophysiological study (n = 47 healthy participants aged between 19-32 yrs) examined whether prediction errors of upcoming pain encoded throughout a delay classical conditioning paradigm (50% reinforcement schedule with a reversal and extinction phase) would alter pain perception (subjective ratings) and spinal nociceptive responses (RIII-reflex) to a noxious electrical shock (US) administered to the right sural nerve (135% of RIII threshold). Visual conditioned stimuli (CS+/CS-) were colored fractal images presented for 2 sec. Learning was assessed with skin conductance responses estimated for each unreinforced CS trial. Prediction errors were computed using a standard Rescorla Wagner model including a constant learning rate. Initial expected values of US occurrence were set at 0.5 and the learning rate was set as a free parameter, estimated using optimisation search algorithms. Multi-level regression analyses revealed that the relationship between prediction errors and spinal pain responses (normalised NFR scores), was significantly increased ($p = .02$) for participants who were consciously aware of CS-US contigencies (n = 24) relative to those who had not explicitly detected CS-US pairings. In contrast, prediction errors impending pain were not related ($p = .84$) to subjective pain evaluations of the US. These results show that outcomes highly discrepant from expectations predict reduced spinally-mediated physiological responses to pain, but only when consciously aware of learning contingencies. This study demonstrates that fear-induced anti-nociception can

be modelled dynamically using basic indices reflecting prediction processes derived from classical learning theories.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01 MH046729

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NIH Grant P50 MH84051

Title: Overexpression of corticotropin releasing hormone in the central nucleus of the amygdala alters metabolism and functional connectivity in non-human primates

Authors: ***A. S. FOX**[1], J. A. OLER[1], D. R. MCFARLIN[1], B. P. GRABOW[2], M. E. OLSEN[2], E. K. BRODSKY[2], R. KOVNER[1], M. K. RIEDEL[1], E. M. FEKETA[1], D. P. M. TROMP[1], R. M. BIRN[1,2], P. H. ROSEBOOM[1], A. L. ALEXANDER[1,2], M. E. EMBORG[2,3], W. F. BLOCK[2], N. H. KALIN[1,3]
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Abstract: Children with an extremely anxious temperament (AT) are at risk to develop anxiety and depressive disorders. Our group has developed a rhesus monkey model of early life AT, and identified its distributed neural substrates, which include the orbitofrontal cortex (OFC), hippocampus, portions of the brainstem, and the central nucleus of the amygdala (Ce). The Ce is essential to the processing of fear and anxiety, is connected to other AT-related regions, and lesions of the Ce are sufficient to reduce AT. The Ce contains a rich mixture of peptides that have the potential to modulate anxiety responses. Of particular interest is corticotropin releasing hormone (CRH), which we have shown to increase AT in young rhesus monkeys by injecting an AAV2 vector into the Ce to increase expression of CRH transcripts (Ce-CRH, see Oler et al., SFN 2014). Injecting a viral-vector that upregulates a particular gene provides an exciting opportunity to demonstrate that increased gene expression in a specific region can cause

alterations in behavior. Here, we combined this approach with multimodal neuroimaging to examine Ce-CRH induced alterations in brain metabolism and connectivity throughout the AT network. This approach allows for unprecedented insights into how molecular alterations within the Ce can result in distributed alterations in processing throughout the primate brain. We assessed brain metabolism using FDG-PET in response to potential-threat, as well as intrinsic connectivity with 'resting' fMRI, both before and again approximately 2 months after surgery in 5 Ce-CRH injected monkeys, and at corresponding times in 5 unoperated controls. Results demonstrate significantly greater post-pre change in Ce metabolism in the Ce-CRH group compared to controls ($p<.01$, uncorrected). Furthermore, whole-brain analyses reveal significant alterations in OFC, hippocampus, and brainstem metabolism, all regions that have been implicated in AT. We examined injection-induced changes in the synchrony of BOLD fluctuations with fMRI, using the bilateral Ce-CRH injection region as a seed for connectivity analyses. Results demonstrated that functional connectivity with Ce significantly decreased in the insular cortex, and increased in a region encompassing portions of substantia innominata and internal globus pallidus ($p<.01$, uncorrected). This work, aimed at understanding the effects of increased CRH in the Ce, will help motivate the development of novel interventions designed to prevent the development of anxiety disorders. This approach begins to elucidate the neural mechanisms by which local gene expression can alter the function of brain-wide networks to influence behavior.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 470.10/VV47

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01 MH046729

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NIH Grant P50 MH84051

Neuroscience Training Program

Title: Distal microstructural alterations resulting from CRH overexpression in the central nucleus of the amygdala of non-human primates

Authors: *D. P. TROMP[1,2], A. S. FOX[1], J. A. OLER[1], B. P. GRABOW[3], M. E. OLSEN[3], E. K. BRODSKY[3], R. KOVNER[1], M. K. RIEDEL[1], E. M. FEKETE[1], R. M. BIRN[1,3], P. H. ROSEBOOM[1], A. L. ALEXANDER[1,3], M. E. EMBORG[3,4], W. F. BLOCK[3], N. H. KALIN[1,4]
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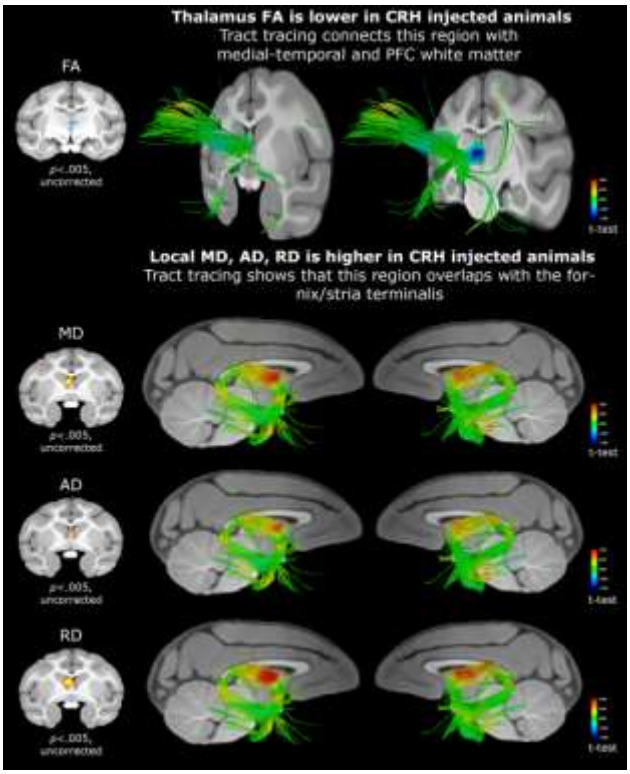
Abstract: Early-life anxious temperament (AT) often leads to debilitating anxiety disorders. Our group has developed a non-human primate model of early-life AT, and identified alterations in metabolism and connectivity of the central nucleus of the amygdala (Ce) as important for this risk. Corticotropin releasing hormone (CRH), which is highly expressed in the Ce, is important in modulating the neural response to stress. To investigate the effects of increased CRH expression in the Ce on the network of brain systems that underlie anxiety, we injected the Ce of non-human primates with an AAV2 vector containing a CRH construct to increase the expression of CRH. In addition to altering function in the infected region, altering local gene expression may fundamentally influence the larger brain network. To investigate how brain microstructure changes as a function of CRH gene overexpression, we collected imaging data from 10 rhesus macaques; 5 before and after Ce AAV2-CRH injections and 5 unoperated controls at similar intervals. Diffusion tensor imaging (DTI) was used in conjunction with fiber tractography to study the influence of Ce CRH overexpression on microstructure as measured with fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Voxelwise analyses revealed reduced FA in the thalamus approximately 3 months after Ce AAV2-CRH injection ($p<.005$, uncorrected). Voxelwise analyses of MD, AD and RD revealed similar group X time interactions, such that the CRH group showed significant increases in each of these diffusivity measures within a cluster encompassing both the fornix and stria terminalis ($p<.005$, uncorrected). Taken together these results indicate that chronically increased Ce CRH expression not only influences behavior and brain function (see Oler et al., & Fox et al., SFN 2014), but also influences long-range connectivity and microstructure. These findings suggest potentially new mechanisms by which chronically increased CRH activity may influence behavior and the vulnerability to develop stress-related psychopathology.



Disclosures: **D.P. Tromp:** None. **A.S. Fox:** None. **J.A. Oler:** None. **B.P. Grabow:** None. **M.E. Olsen:** None. **E.K. Brodsky:** None. **R. Kovner:** None. **M.K. Riedel:** None. **E.M. Fekete:** None. **R.M. Birn:** None. **P.H. Roseboom:** None. **A.L. Alexander:** None. **M.E. Emborg:** None. **W.F. Block:** None. **N.H. Kalin:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01 MH046729

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Title: Targeted gene delivery using MRI-guided surgery to alter anxiety in non-human primates

Authors: *J. A. OLER[1], A. S. FOX[1], B. P. GRABOW[2], M. E. OLSEN[2], E. K. BRODSKY[2], R. KOVNER[1], M. K. RIEDEL[1], E. M. FEKETA[1], P. H. ROSEBOOM[1], A. L. ALEXANDER[1,2], M. E. EMBORG[2,3], W. F. BLOCK[2], N. H. KALIN[1,3]
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Abstract: Children at risk to develop anxiety and depressive disorders often show increased behavioral inhibition and anxious temperament (AT). We developed a non-human primate model of AT, demonstrating that the central nucleus of the amygdala (Ce) is a critical structure underlying the risk to develop anxiety and depression. FDG-PET studies in monkeys demonstrate that the Ce is a core component of the neural network predictive of AT. Furthermore, neurotoxic lesions of the Ce that attenuate AT provide causal evidence for Ce involvement in early-life anxiety. Corticotropin releasing hormone (CRH) is a peptide that mediates the expression of stress reactivity within the HPA axis, as a hormone, and also a neurotransmitter in the circuit underlying AT. Here we utilized viral vector technology to overexpress CRH in the Ce of young rhesus monkeys to alter AT. We studied 10 monkeys, 5 of which received bilateral Ce injections (24µl/side) of an adeno-associated virus with a CRH construct (AAV2-CRH). The other 5 animals served as non-operated controls. The AAV2-CRH was mixed with the contrast agent gadolinium (Gd, 0.66 mM), and was administered using convection enhanced delivery. To ensure precise localization of the target, the infusion was performed in the MRI allowing for real-time monitoring of the infusion. This method was first performed in one pilot animal that at post-mortem demonstrated selective and high levels of CRH expression. To estimate the diffusion of AAV2-CRH, we examined the overlap of MR-visible Gd in standard space. Results demonstrated the precision of the MRI-infusion method, as all 5 subjects had detectable Gd within an overlapping Ce-region. AT was assessed before surgery and again approximately 2 months after for Ce-CRH animals and at similar intervals for the controls using the no-eye-contact (NEC) condition of the human intruder paradigm. During NEC the monkey is placed in a cage and a human enters the room and stands 2.5m from the animal without making eye contact. Freezing, coo vocalizations and plasma cortisol levels in response to NEC were measured, and AT was calculated as the mean of these 3 z-scored variables. We examined the post-pre change in AT. Animals with CRH overexpression in the Ce demonstrated a significant increase in AT ($p<.05$, one-tailed). These results demonstrate the potential for gene delivery in primate models to explore novel treatment strategies for refractory psychiatric illness. The findings demonstrate the importance of altered CRH function in primate anxiety, suggesting a similar role in humans. Future work will use this method to deliver viruses containing constructs relevant to amygdala function and neuropsychiatric illnesses.

Disclosures: J.A. Oler: None. **A.S. Fox:** None. **B.P. Grabow:** None. **M.E. Olsen:** None. **E.K. Brodsky:** None. **R. Kovner:** None. **M.K. Riedel:** None. **E.M. Feketa:** None. **P.H. Roseboom:** None. **A.L. Alexander:** None. **M.E. Emborg:** None. **W.F. Block:** None. **N.H. Kalin:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Title: Pain and decision style affects performance on emotional decision-making

Authors: *B. FURL, F. S. RASSU, M. W. MEAGHER
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Abstract: Persistent pain can result in emotional dysfunction, cognitive impairment and reduced quality of life. However, pain's effect on complex decision-making, which relies on cognitive and emotional influences, is not well understood. In this study, we examined the effect of thermally enhanced, capsaicin-induced pain on the Iowa Gambling Task, a computerized card game developed to study complex, emotional decision-making. Healthy undergraduates completed the task either during pain induction (n= 21) or without pain (n = 18). Skin conductance level was monitored, and participants completed self-report surveys measuring aspects of decision-making style and reward responsiveness. We hypothesized that pain would lead to impaired performance on the gambling task, relative to controls, and this would be associated with the development of differential anticipatory skin conductance as the advantageous decision strategy was learned. We also expected that lower self-reported impulsivity and greater rational decision-making style would predict enhanced performance on the gambling task. As hypothesized, those in pain performed poorly relative to controls ($t(37) = 2.0$, $p = .05$). In particular, pain tended to enhance selection of deck B ($t(37) = -2.2$, $p = .03$) which is the disadvantageous deck that results in the highest frequency of rewards. As expected, self-reported rational decision-making style was associated with enhanced performance ($r = .59$, $p = .02$), but only in the control condition. Surprisingly, experiential/intuitive decision-making style predicted enhanced task performance in the control condition ($r = .53$, $p = .02$) and impaired performance ($r = -.53$, $p = .02$) in the pain condition. Reward responsiveness also predicted impaired performance ($r = -.44$, $p = .02$) in the pain condition. Analysis of the skin conductance responses is ongoing. These findings indicate that pain impairs complex, emotional decision-making and that the impairment may be enhanced by high reward responsiveness and influenced by decision-making style.

Disclosures: B. Furl: None. **F.S. Rassu:** None. **M.W. Meagher:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: MRC Centenary Award RG68925 to YM

EPSRC Grant S10829 to DF

Title: Undifferentiated physiological responses to safety and unpredictable threat are associated with high trait anxiety and lower emotional resilience in competitive sport

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Abstract: Competitive sport performers are regularly challenged to achieve high levels of performance under intense stress and anxiety, and research in competitive sport may provide novel insights into emotion regulation. The current study investigated threat processing in sport performers using a translational test with a well-characterized neural and neurochemical basis (the Neutral-Predictable-Unpredictable threat test, NPU; Schmitz and Grillon, 2012, Nat Protoc 7: 527-532). We compared physiological and subjective responses to cued fear and contextual anxiety, and examined individual differences related to trait anxiety and resilience in the sport domain. Facial EMG startle responses were assessed in 32 student performers from a range of team and individual sports (17 women, 15 men; age 23 ± 0.8) who were separated into low and high trait-anxious subgroups based on the Sport Anxiety Scale-2. Responses during presentation of visual cues and inter-cue intervals ("context") were examined across three conditions: N (safe), P (mild electric shock only during cue) and U (shock at any time). Overall, startle responses and subjective anxiety ratings were significantly ($p < 0.001$) potentiated by both cued fear ($P_{cue\text{-}context}$) and contextual anxiety ($U_{context}$-$N_{context}$). The low-anxious group differentiated between P and U contexts, displaying potentiated startle only during the latter; in contrast, the high-anxious group did not show such differentiation, displaying elevated startle during both (condition x group interaction $F_{(2,48)} = 4.47$, $p < 0.05$). This reflected their inability to use $P_{context}$ as a period of explicit safety, together with their apparent response bias to the non-predictive U_{cue} relative to the $U_{context}$ ($U_{cue\text{-}context}$ *vs.* trait anxiety: Pearson r = 0.54, $p < 0.01$). Higher levels of trait anxiety were paralleled by a less resilient psychological profile on the Sport Resilience

Scale. The findings demonstrate that NPU startle responses are sensitive to non-clinical differences in trait anxiety, extending previous work in clinically-anxious groups. Impaired use of safety signals, and anxious responses to neutral cues during threat in high trait-anxious individuals are in line with anxiety theories and recent findings in non-human primates in our laboratory (Mikheenko *et al.,* 2012, SfN 422.06, Shiba *et al.,* 2014, Front Behav Neurosci 8: article 137). Their contribution to low emotional resilience may inform strategies for developing resilience in high-performance contexts.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: NIH Grant MH091864

Title: Early maternal deprivation accelerates amygdala-based fear learning in humans

Authors: *J. A. SILVERS[1], D. S. LUMIAN[3], L. GABARD-DURNAM[3,2], D. GEE[3], B. GOFF[3], D. S. FARERI[3,2], C. CALDERA[3], J. FLANNERY[4], E. TELZER[5], K. HUMPHREYS[3], N. TOTTENHAM[3,2]
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Abstract: Early life stress can profoundly influence fear behavior in childhood and beyond. As members of an altricial species, there is perhaps no early life stressor more disruptive for humans than maternal deprivation, which has been shown to accelerate amygdala development and associated fear learning in rodents following maternal deprivation. However, it has yet to be demonstrated whether maternal deprivation enhances the development of amygdala-based fear learning in humans, who typically remain dependent on caregivers longer than any other species. To address this issue, we compared 27 previously institutionalized (PI) children (18F/9M; 5-11 years) to 52 comparison children who had never experienced institutional care (33F/19M; 5-11 years). To assess amygdala-mediated fear learning, participants completed a conditioning paradigm while undergoing functional neuroimaging wherein visual cues were paired with either

a neutral or aversive tone (CS- and CS+, respectively). Compared to same-aged controls, PI children showed exaggerated amygdala responses to the conditioned stimuli and potentiated fear learning. These data build on existing evidence showing greater amygdala reactivity in children following maternal deprivation and show that this hyperactivity is associated with group differences in learning during childhood. These findings suggest ontogenetic acceleration of human amygdala-based fear learning following maternal deprivation.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: UNC Provost Award

Stress and Motivated Behavior Institute

Title: Anxiety vulnerable individuals exhibit reduced acoustic startle response

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Abstract: Prior work with individuals self-reporting behavioral inhibition has revealed enhanced eyeblink conditioning to a tone CS and US air puff in a variety of training protocols. One underlying mechanism for this enhanced associative learning could be a heightened responsivity to the stimuli used. In previous eyeblink conditioning studies, there were no differences in reflexive responding to the US air puff. However, a rodent model of inhibited temperament (i.e., Wistar-Kyoto) has indicated enhanced acoustic startle response (ASR) as compared to a non-inhibited (i.e., Sprague Dawley) strain (Avcu et al., 2013). In the work reported here, we tested the ASR of behaviorally inhibited and non-inhibited individuals (as measured by the Adult Measure of Behavioral Inhibition). We investigated possible physiological differences in responsivity to auditory stimuli in anxiety vulnerable individuals that may underlie our previous

findings with classical eyeblink conditioning. Ninety five college-aged undergraduate students voluntarily participated in the study in exchange for research credit for psychology coursework. All participants completed inventories on personality, stress, and history of head injury. We measured ASR through eyeblink-related EMG activity. The session consisted of 180 seconds of pre and post baseline monitoring. The acoustic stimuli were a 50 ms white noise bursts (82, 92, or 102 dB) presented pseudo-randomly eight times each for a total of 24 trials. Data were grouped and analyzed based on a median split of the AMBI score. As expected, ASR increased as the volume of the auditory stimulus increased. ASR also habituated across the eight trials for each volume. Overall, behaviorally inhibited individuals exhibited a reduced acoustic startle response to auditory stimuli as compared to non-inhibited individuals. These findings also fit with recent findings of reduced cortisol levels in behaviorally inhibited individuals (Kostek et al., 2014). Behaviorally inhibited individuals may be more sensitive to stressful stimuli, but long term activation of the hypothalamic pituitary axis (HPA) may result in down regulation of the HPA response via negative feedback, thus resulting in reduced ASR. The current findings along with prior findings of no differences in reflexive responding to the air puff stimulus support the hypothesis that he facilitated learning of anxiety vulnerable individuals is based on enhanced associative learning rather than higher responsivity to the conditioning stimuli.

Disclosures: **T. Allen:** None. **M. Sprycha:** None. **R.J. Servatius:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Support: DFG Grant SFB TRR 58, B05

Title: Fear that face - Studies on the electrocortical facilitation of faces in (social) conditioning

Authors: *M. J. WIESER
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Abstract: Sensory facilitation of cues that predict harm is a useful mechanism for efficient detection of threat in the environment. Recent studies employing steady-state visual evoked potentials demonstrated that low-level visual cues previously paired with aversive events lead to enhanced sensory gain in early visual cortex. From a social affective neuroscience perspective,

faces may constitute an interesting social cue, which may lead to different reactions and impressions due to simple associative learning mechanisms. Therefore, in several studies we investigated whether faces when paired with different aversive events lead to enhanced electrocortical responses. Normally, the unconditioned stimulus (US) in fear conditioning paradigms consists of highly aversive stimuli such as electric shocks and loud aversive noise. While these have been shown to be highly effective in eliciting fear responses, one may question their ecological validity as humans rarely encounter them in daily life. Thus, we used nonverbal social gestures and auditory verbal feedback as social US in two social conditioning paradigms together with steady-state evoked potentials (ssVEP) methodology to further elucidate the role of social US in discriminative fear learning and its electrocortical correlates. Furthermore, its potential modulation by social anxiety was investigated. In a third study, social features in faces (facial expressions and gaze direction) were manipulated as predictive cues. Amplitudes of the face-evoked ssVEP revealed larger cortical mass activity in response to faces both paired with negative and positive compared to neutral hand gestures indicating successful affective learning and concomitant short-term plasticity in visual cortex depending on the learning experience. In the second experiment with auditory feedback as US, this effect was replicated in low socially anxious participants, but high socially anxious subjects did not differentiate cortically between the three types of CS faces. Overall, the results point at enhanced sensory gain elicited by threat-predictive faces. As the results of the third experiments show, gaze cues seem to play a subordinate role, but may lead to different time courses in visuocortical learning, as single-trial analysis of ssVEP amplitudes revealed. Together, these results point at the significance of learning mechanisms in social contexts, but suggest that the role of cues such as facial expressions is less prominent in transient cortical plasticity in early visual areas. In addition, the ssVEP methodology also offers a promising avenue for investigating trial-by-trial cortical dynamics in aversive learning.

Disclosures: M.J. Wieser: None.

Poster

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Support: NSERC Grant 203710-11

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Title: Functional neural responses to threat processing in relation to morning cortisol changes among temperamentally shy adults

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Abstract: Temperamental shyness is a stable personality trait characterized by affective, behavioral, and psychophysiological markers of stress vulnerability. Although temperamental shyness has been linked to neuroendocrine dysregulation across development, including high and low baseline cortisol levels (Schmidt et al., 2007), no studies have examined the neural mechanisms underlying this relation in humans. We examined whole-brain patterns of neural responses as a function of changes in morning salivary cortisol peaks during the processing of two types of threat in young adults selected for high (*n*= 12) and low (*n*= 12) shyness. Participants were scanned at 3T while they viewed pairs of faces (e.g., angry/angry or angry/neutral) and indicated whether they were same or different. Peak response of morning cortisol was derived from the difference between the awakening and 60 min post awakening saliva samples across three mornings. Neuroimaging and cortisol data were analyzed using Partial Least Squares (PLS; McIntosh & Lobaugh, 2004). PLS identified no reliable patterns of neural activation in the nonshy group, but within the shy group, separate sets of corticolimbic regions emerged as a function of varying cortisol levels in response to both types of threat (*p*= .05). Shy individuals with relatively lower morning cortisol peaks displayed higher activation in the left amygdala, right posterior cingulate, insula, bilateral inferior, medial and middle frontal gyri. In contrast, shy individuals with relatively higher morning cortisol peaks displayed higher activation in the bilateral rostral anterior cingulate. Relative to nonshy adults, individual differences in morning cortisol changes are associated with differential engagement of brain regions to both types of threat among shy adults. This neuroendocrine variation in temperamental shyness may reflect different cognitive control and affective regulation during threat processing. Similar to patterns of reduced cortisol responses evident in other stressed profiles, the increased amygdala activation in shy adults with reduced morning cortisol responses potentially contribute to increased corticotropin-releasing hormone levels and adaptation of biological systems in response to social anxiety across development. References McIntosh, A.R., & Lobaugh, N.J. (2004). Partial least squares analysis of neuroimaging data: applications and advances. *Neuroimage, 23*, 250-63. Schmidt, L.A., Santesso, D.L., Schulkin, J., & Segalowitz, S.J. (2007). Shyness is a necessary but not sufficient condition for high salivary cortisol in 10 year-old children. *Personality and Individual Differences*, *43*, 1541-51.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Modelling behavioural inhibition in a human approach-avoidance task

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Abstract: Approach avoidance conflict tasks are common rodent models of anxiety, in which an animal is motivated both to approach a spatial location or perform an action, and to avoid it. These tasks have recently been extended to encompass human behaviour. Behavioural inhibition - delaying a decision to approach or to avoid - is observed in these ethological models and has been proposed to reflect risk assessment, or to be due to decision difficulty. This implies that inhibition should not delay a motor action once all information has been gathered and a decision has been taken. Here, use a cost minimisation model to analyse a situation in which decision and motor action are decoupled. In this task, human players leave a "safe place" to obtain monetary tokens which appear randomly and decay exponentially. A looming "predator" might wake up and catch the player and all previously collected tokens at any point in time with a flat hazard function. In this situation, all information required for the decision is known before a token appears. Minimising cost in this task mandates minimising motor response latency. Yet, if the player mistakenly assumes a biologically plausible temporal correlation of threat and reward, it can be cost-minimising to delay a motor action. If this is the case, we show that the optimal delay increases both with increasing threat probability and with increasing stakes. Predictions from this model are tested in two behavioural experiments with different graphical set ups. In both tasks, response latency increases with both increasing threat probability and increasing potential loss. This supports a model in which a temporal coupling of reward and threat is subjectively assumed. To conclude, our model and data suggest that behavioural inhibition might be a biologically adaptive behaviour under prior assumptions about temporal coupling of threat and reward.

Disclosures: D.R. Bach: None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Strategic Research Program for Brain Sciences, Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Title: A model of amygdala-mPFC interactions for large resistance to extinction of partially reinforced fear memory

Authors: *Y. LI[1], S. ISHII[2], H. NAOKI[3]
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Abstract: Extinction of fear memory stored in amygdala is regulated by inhibition of amygdala activity through medial prefrontal cortex (mPFC). Resistance to extinction is relatively larger after partial reinforcement schedule of fear conditioning, in which conditioned stimulus (CS) is partially paired with unconditioned stimulus (US), compared with continuous reinforcement schedule. However, how the amygdala and mPFC encode the partial reinforcement schedule remains elusive. To this end, we developed a neural circuit model that includes interactions between subpopulations of neurons in amygdala and mPFC. In the computer simulation, we reproduced behaviors of the amygdala and mPFC activities as conditioned response in the extinction after not only full but also partial schedule. Based on the result, we proposed that plasticity of synaptic input to the mPFC is modulated by surprise-like signal, which expressed by balance between activities of the neuron subpopulations in amygdala and mPFC. Moreover, our model provides a prediction of therapy treatment to eliminate the resistant fear memory. Therefore, our model shed light on neural circuit-level understanding of large resistance to extinction.

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Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Topic: F.03. Motivation and Emotion

Title: Theta activity in Human approach-avoidance behaviour

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Abstract: Common non-human animal models of anxiety build on conflict between approach and avoidance motivation, as in elevated plus maze and open-field test. A body of rodent studies have demonstrated the role of ventral hippocampus in arbitrating approach-avoidance conflict, and recent work has extended this to its human homologue, the anterior hippocampus. In these tasks, rodent hippocampus shows increase in power and frequency of theta band oscillations. Here, we probe the functional homology of electrophysiological responses in human hippocampus during approach-avoidance behaviour, using magnetoencephalography (MEG). Emulating rodent paradigms drawing on operant conflict tests, we developed a human approach-avoidance task, embedded in a computer game. In each trial, participants could make a response to collect a monetary token that served as approach incentive. To provide avoidance motivation, a sleeping "predator" present in each trial could catch the human player, leading to loss of tokens. The predator had three different levels of threat, corresponding to wake up probability. We report a behavioural study with 20 participants and a MEG study with 25 participants, playing the same game with 648 and 540 trials, respectively. In the behavioural task, participants collected more tokens and had faster reaction times in low threat and low loss situations, while they showed avoidance (no response) and behavioural inhibition (longer RTs) when threat or potential losses were higher. In MEG, we observed increased induced theta (4-6 Hz) responses across all threat levels in medial temporal lobe within 1s of the token appearance. Evoked responses in parieto/temporal areas distinguished between threat levels. To summarize, our results suggest that human hippocampal theta activity increases during approach/avoidance conflict. This establishes homology to rodent work and paves the way towards probing the underlying functional role of this oscillatory activity.

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Poster

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Dana Foundation Grant

Title: Distinct medial prefrontal mechanisms associated with perceived threat biases and hyperarousal symptoms in OEF/OIF veterans

Authors: *D. W. GRUPE[1], J. B. NITSCHKE[2], R. J. DAVIDSON[1]
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Abstract: Combat-related posttraumatic stress disorder (PTSD) afflicts hundreds of thousands of veterans of Operations Enduring Freedom and Iraqi Freedom (OEF/OIF). Heterogeneity in the nature of PTSD suggests distinct biological mechanisms for the disorder, yet many neuroimaging studies adopt a categorical groups approach that fails to adequately account for between-subject variance. Greater insight into the neurobiology of PTSD can be gained by relating brain imaging measures to individual differences in traits or specific symptoms that bear a closer relationship to distinct biological mechanisms. To this end, we collected fMRI and DTI data from 50 male OEF/OIF veterans with a wide range of PTSD symptom severity. Using an instructed threat conditioning paradigm, we investigated anticipatory brain activation under conditions of unpredictable threat and safety in the medial prefrontal cortex (mPFC) and amygdala, two regions frequently implicated in the pathology of PTSD. We regressed anticipatory activation in these regions on 1) individual differences in hyperarousal and hypervigilance symptoms, and 2) biased perceptions of threat while deployed, relative to actual exposure to combat trauma. Hyperarousal symptoms were associated with less differentiated activation for safe and threat anticipation in the ventral mPFC (vmPFC) and basal amygdala. Furthermore, biased perceptions of threat while deployed were positively related to anticipatory threat activation in the pregenual anterior cingulate cortex (pACC). Hyperarousal symptoms were uncorrelated with perceived threat biases, and each of the above correlations remained significant while controlling for the other measure. Underscoring the independence of these two measures, we found opposite

relationships with prefrontal white matter structural integrity, as indexed by fractional anisotropy (FA) values. Hyperarousal symptoms were positively correlated with FA values in the uncinate fasciculus, whereas perceived threat biases were negatively correlated with FA values in the pACC. These data provide evidence for independent biological mechanisms associated with biased perceptions of threat and hyperarousal symptoms in OEF/OIF veterans, and provide groundwork for future research that may lead to improved nosology and individualized treatment of PTSD.

Disclosures: D.W. Grupe: None. **J.B. Nitschke:** None. **R.J. Davidson:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 470.22/VV59

Topic: F.03. Motivation and Emotion

Title: Effects of paced breathing on cognitive flexibility during social-evaluative stress

Authors: *B. J. FERGUSON[1], J. HADLEY[1], W. SNYDERS[1], D. Q. BEVERSDORF[2]
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Abstract: Previous studies have shown that propranolol, a beta adrenergic antagonist, can reduce the negative effects of stress on problem solving abilities. Furthermore, a recent investigation using meditation training showed decreases on social evaluative stress without pharmacological intervention, and a component of meditation training is slowed breathing. Taken together, it may be possible to enhance cognitive flexibility, or the ability to inhibit a dominant response when it represents a non-optimal or inappropriate solution to a problem, by engaging in slowed breathing prior to a social evaluative threat. For example, the use of paced breathing, a technique used to slow the rate of breathing in an experimental setting, has been shown to increase heart rate variability, an indication of increased parasympathetic nervous system tone. Furthermore, increased parasympathetic nervous system tone is the result of administration of propranolol, which has already shown positive effects on problem solving abilities during stress. This goal of this project is to better understand if paced breathing can be implemented to alleviate problem solving deficits caused by stress. If relaxation techniques are as effective as propranolol in alleviating problem solving deficits, the future of this project can possibly benefit those who suffer from test or public speaking anxiety. In this ongoing project, we hypothesize that paced

breathing prior to social-evaluative threat will lead to increased performance on measures of cognitive flexibility. Likewise, these effects will covary positively with heart rate variability, an indication of increased parasympathetic influence, and negatively with heart rate and blood pressure. The results will be discussed in terms of the effects of paced breathing on cognitive flexibility, a necessary component of creativity.

Disclosures: B.J. Ferguson: None. **J. Hadley:** None. **W. Snyders:** None. **D.Q. Beversdorf:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 470.23/VV60

Topic: F.03. Motivation and Emotion

Title: Human primary auditory cortex encodes threat-predicting information of complex but not simple sounds during fear conditioning

Authors: *M. STAIB, D. R. BACH
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Abstract: Learning to predict an aversive event from neutral stimuli is experimentally modelled in fear conditioning. In delay conditioning paradigms, a neutral stimulus (conditioned stimulus, CS+) contingently co-terminates with an aversive stimulus (unconditioned stimulus, US) which after a few trials induces a conditioned response (CR) during anticipation of the US. In animal studies the amygdala has been identified as brain structure required for this type of learning when simple sine tones are used as CS. On the other hand, rodents with lesioned primary auditory cortex (PAC) still show a CR, suggesting the PAC may not be required for fear learning. Recently this view has been challenged by studies using complex sounds as CS, comprised of multiple frequencies and temporal patterns. In these studies, fear learning was impaired after PAC inhibition. This suggests that PAC is necessary for fear learning from complex sounds, either in order to extract information from the individual sounds and forward them to the amygdala where CS/US association is learned, or to directly form a CS/US association. In the present study, we investigate the role of PAC for fear learning in humans. Twenty healthy subjects underwent a differential delay fear learning paradigm in a reinforcement context where the CS+ is probabilistically paired with an unpleasant electrical stimulation while the CS- is always presented alone. In a non-reinforcement context, different sets of complex and

simple sounds were always presented alone (neutral sounds, NS). High-resolution functional MRI (fMRI) was recorded to measure blood-oxygen-level dependent (BOLD) signal associated with the presentation of sounds. Skin conductance response (SCR) was recorded simultaneously to estimate the conditioned sympathetic response. We employed multivariate pattern analysis (MVPA) to discriminate BOLD patterns elicited by CS+/CS-, or two different NS, in the superior temporal sulcus including PAC. Classification performance in the STS was higher for complex CS than for simple CS or NS, as shown by an interaction complexity x reinforcement context. Our results indicate that PAC encodes differences between complex (but not simple) CS+ and CS- over and above physical differences between the two sounds. This suggests that the role of the PAC extends beyond sound processing and is in keeping with a model of a distributed fear learning network in the human brain, involving both PAC and amygdala for establishing fear memory.

Disclosures: **M. Staib:** None. **D.R. Bach:** None.

Poster

470. Fear and Anxiety: Human and Nonhuman Primates

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 470.24/VV61

Topic: F.03. Motivation and Emotion

Title: Specific connectivity profiles for deep and superficial amygdala nuclei in humans

Authors: *A. ABIVARDI, D. R. BACH
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Abstract: *Objective:* Amygdalo-cortical pathways have been studied thoroughly in non-human animals using qualitative and semi-quantitative tracing techniques. Probabilistic tractography furnishes a quantitative method for analyzing human connectivity *in vivo*. Systematic investigations of amygdalo-cortical networks in humans are still missing. Here we provide detailed cortical connectivity profiles for deep and superficial amygdala nuclei. *Methods:* Probabilistic tractography was performed in 8 individuals, using diffusion-weighted magnetic resonance images. We relied on a previously established amygdala parcellation into deep and superficial nucleus groups based on two distinct cortical areas. Using these nucleus groups as seed regions, we determined connections to the entire cortex, segmented into thirty-five areas for each hemisphere based on individual T1-weighted images. *Results:* Parahippocampal gyrus, entorhinal cortex, fusiform gyrus, insula and the lateral occipital cortex, in descending order,

showed highest probability of connecting with the amygdala. Marked differences in connectivity profiles for deep and superficial clusters were found. In particular, we observed stronger connections for deep clusters to the entorhinal and the pericalcarine cortex as well as stronger connections for superficial clusters to the inferior parietal and the lateral occipital cortex. *Conclusion:* Several amygdalo-cortical pathways are proposed to be implicated in various neurological and neuropsychiatric disorders, e.g. connectivity to superior temporal sulcus and fusiform gyrus in autism or to the visual cortex in schizophrenia. Therefore a sound and comprehensive understanding of amygdala connectivity in humans is needed. We propose that structural connectivity profiles may be used as a guide for functional and pathophysiological neuroimaging studies.

Disclosures: A. Abivardi: None. **D.R. Bach:** None.

Poster

471. Invertebrate Motor Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 471.01/VV62

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: HHMI

Title: Multiple stages of functionally synergistic multimodal convergence

Authors: *M. ZLATIC[1], T. OHYAMA[2], C. SCHNEIDER-MIZELL[3], J. TRUMAN[2], R. FETTER[2], R. FRANCOVILLE[2], A. CARDONA[2]
[1]HHMI: Janelia Farm Res. Campus, Ashburn, VA; [2]HHMI Janelia Farm, Ashburn, VA; [3]HHMI Janelia Farm, Asburn, VA

Abstract: Information carried by a single sensory modality is noisy and may be insufficient to unambiguously detect stimuli. Nervous systems have evolved ways of combining information from multiple modalities to decrease uncertainty and ambiguity, but the way in which they do so is still unclear. A prerequisite for understanding the logic of multimodal integration is identifying the structural basis of multimodal convergence - identifying at which levels in the circuit and onto which neurons do modalities converge. We investigate the principles of multimodal integration in *Drosophila* larva where we can combine neural manipulation in freely behaving animals, functional imaging and large-scale EM reconstruction to identify the behaviorally relevant multisensory neurons and the circuit architecture of multimodal convergence onto these

neurons. In larvae attack of a predator can evoke an energetically costly escape response: rolling. In principle, predator attack can stimulate two sensory modalities: nociceptive and mechanosensory. We showed that combining mechanosensory with nociceptive stimulation increases the probability of escape response. In a large-scale screen we identified central neurons whose thermogenetic activation was sufficient to evoke the escape response. We reconstructed the behaviorally relevant neurons and their pre and postsynaptic partners (total 150.4 cm of cable and 557 neuronal arbours) in a serial section TEM volume that comprises the entire larval nervous system. This revealed a complex circuit that mediates larval escape response, in which multimodal convergence starts at the earliest stages of sensory processing and continues at multiple subsequent levels of the network. 1st order multisensory projection neurons (basins), necessary and sufficient for rolling, receive direct synaptic inputs from nociceptive neurons on one dendritic branch and mechanosensory neurons on another and respond synergistically to the combination of stimuli. Basins mediate rolling by (indirectly) activating a single pair of roll-command-like neurons (gorgoro) in the motor domain of the nerve cord, also necessary and sufficient for rolling. We identify distinct anatomical pathways from basins to gorogoro: via the nerve cord and via the brain. Parallel unisensory pathways re-converge with the multisensory pathways at multiple nodes downstream of the basins. Combining sensory inputs at multiple levels in the network and having continuous feedforward and feedback interactions between the unisensory and multisensory pathways enables multiple independent steps of noise reduction and may be a general principle for multimodal integration.

Disclosures: **M. Zlatic:** None. **T. Ohyama:** None. **C. Schneider-Mizell:** None. **A. Cardona:** None. **R. Fetter:** None. **R. Francoville:** None. **J. Truman:** None.

Poster

471. Invertebrate Motor Circuits

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Program#/Poster#: 471.02/VV63

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Mapping sensorimotor networks in *Drosophila* using optogenetics and two-photon calcium imaging

Authors: *R. FRANCONVILLE, V. JAYARAMAN
Janelia Farm Res. Campus, Ashburn, VA

Abstract: The central complex, a prominent structure of insect central brains, is thought to play an important role in high-level motor control, most notably during sensory-driven orientation [1, 2]. Although considerable attention has focused on visual maps that constitute some of the inputs to the central complex [3,4], the precise nature of the information exiting the structure is less well understood, as are the neural pathways linking the structure to the motor system. To fill this gap, we used a coarse functional connectivity mapping approach to uncover the identity of neurons constituting output channels of the central complex. We took advantage of the multiple genetic expression systems and the large driver line collections available in *Drosophila* to express a neural activator, CsChrimson or P2X2, in potential presynaptic neurons and the calcium sensor GCaMP6 in potential postsynaptic neurons. We further refined the protocol and combined it with pharmacology to reliably establish polarity of the connection (excitatory or inhibitory). We find that the different classes of central complex "projection neurons", or columnar neurons, contact different subsets of neurons local to the lateral accessory lobe (a neuropile associated to the central complex), which in turn relay information to various parts of the brain. To understand how the parallel channels and interneuron motifs we discovered are related to the fly's motor activity, we are now recording from populations of output neurons in walking animals. We hope that these results, together with existing descriptions of sensory maps within the central complex, will provide a framework to study the sensorimotor computations performed by this intriguing structure. [1] Neuser, K., Triphan, T., Mronz, M., Poeck, B., & Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. Nature, 453(7199). [2] Guo, P. and Ritzmann, R. E. (2013). Neural activity in the central complex of the cockroach brain is linked to turning behaviors. J. Exp. Biol., 216(Pt 6). [3] Heinze, S. and Homberg, U. (2007). Maplike representation of celestial E-vector orientations in the brain of an insect. Science, 315(5814). [4] Seelig, J.D. and Jayaraman, V. (2013). Feature detection and orientation tuning in the *Drosophila* central complex. Nature, 503(7475).

Disclosures: R. Franconville: None. **V. Jayaraman:** None.

Poster

471. Invertebrate Motor Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 471.03/VV64

Topic: F.04. Neuroethology

Title: Organization of descending interneurons in *Drosophila*

Authors: *S. NAMIKI[1], M. DICKINSON[2], A. WONG[1], G. RUBIN[1], W. KORFF[1], G. CARD[1]
[1]HHMI Janelia Farm Res. Campus, Ashburn, VA; [2]Univ. of Washington, Seattle, WA

Abstract: A population of descending interneurons (DNs) connect the brain and the thoracico-abdominal ganglion (TAG), and are thought to be a bottleneck of information flow in the nervous system. Using the Janelia and Vienna databases of GAL4-driven neural expression patterns in the fruit fly, *Drosophila* melanogaster, we conducted a systematic survey and analysis of DNs in this species. We identified 181 DNs (at least 90 different types) among a total number of about 350-400, as measured with photoactivatable GFP experiments. DNs send axonal projection within the TAG, including neck motor (44% of total), flight (43%), haltere (40%), and leg neuropils (23%). DNs often project to the same area in the TAG, suggesting the presence of functional map for motor command. We did not find any DNs that had innervation to the higher order centers such as the central complex and mushroom body. Our survey also indicates the conservation of basic anatomical features among insect species, suggesting the existence of ground pattern in the organization of descending motor pathway. We are currently working to identify the function of these DNs using genetic activation techniques with split-GAL4 lines, which label specific subpopulation of DNs.

Disclosures: S. Namiki: None. **M. Dickinson:** None. **A. Wong:** None. **G. Rubin:** None. **W. Korff:** None. **G. Card:** None.

Poster

471. Invertebrate Motor Circuits

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Program#/Poster#: 471.04/VV65

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: HHMI

Title: Behavior and circuit analysis of sequence generation during grooming

Authors: *J. H. SIMPSON, P. CHUNG, R. FRANCONVILLE, S. HAMPEL, P. RAVBAR, A. M. SEEDS
HHMI Janelia Farm Res. Campus, ASHBURN, VA

Abstract: How the nervous system produces complex innate motor sequences is poorly understood. We are studying the algorithmic and neural circuit basis of sequence generation in

the context of grooming behavior in *Drosophila*. We first establish that fly grooming behavior is composed of discrete motor subroutines that remove dust from different body parts. These subroutines are executed in an order dictated by sensory cues and a hierarchy that prioritizes certain body parts over others. We use large-scale analysis of grooming behavior data and computational modeling to propose what this method of sequence production may actually be optimal for. In contrast to fixed action patterns or behaviors in which the precision of the sequence is essential, grooming appears to be more flexible, potentially permitting more efficient responses to different sensory experiences rather than removing all the dust in the shortest time. We use behavioral genetics to uncover the logic flies use to compare sensory inputs between different body parts, and the rules by which one motor program suppresses execution of others. Finally, we identify various sensory neurons and interneurons capable of initiating specific grooming programs, and link them into potential neural circuits by mapping their functional connectivity using a combination of optogenetics and two-photon calcium imaging. Our work combines manual and automatic behavioral analysis, genetic manipulation of specific neural populations, imaging of neurons and their activity, and computational modeling to gain insights into how the nervous system executes motor subroutines in flexible sequences appropriate for the task.

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Poster

471. Invertebrate Motor Circuits

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Topic: F.04. Neuroethology

Support: NIMH IRP ZIA- MH002800-11

FONDECYT 1141278

NIH R01 NS021749

Title: Neural and molecular determinants of the circuit underlying molting behaviors in *Drosophila*

Authors: F. DIAO[1], F. DIAO[1], J. SHI[2], W. MENA[3], B. MARK[3], D. PARK[2], P. TAGHERT[2], J. EWER[3], *B. H. WHITE[1]
[1]Labor Molec Bio, NIMH, BETHESDA, MD; [2]Washington Univ., St. Louis, MO; [3]Cinv, Univ. Valparaiso, Valparaiso, Chile

Abstract: Ecdysis Triggering Hormone (ETH) plays an essential role in organizing the behavioral aspects of each molt in insects by inducing a motor program known as an "ecdysis sequence." The neuronal basis of ecdysis sequences has been best studied at the pupal molt in *Drosophila* where ETH signaling progressively activates ensembles of peptidergic neurons known to express the A isoform of the ETH receptor (ETHRA). Because ecdysis sequences at different developmental stages differ in motor output, the neural network underlying ecdysis is likely modified during development, but it is not known whether these modifications include changes in the pattern of ETH-signaling. To help answer this question, we have identified and characterized ETH targets using a novel technique that has allowed us to gain genetic access to all cells that express the ETHR gene or to subsets of cells that more selectively express one of the two ETHR isoforms. We find that the two ETHR isoforms, and the cells that express them, play distinct roles in ecdysis at different developmental stages. Consistent with the phenotype of ETH null mutants, ETHR gene knockdown results in lethality at larval ecdysis. Similarly, suppression of activity in ETHRA-expressing neurons also causes 100% lethality at larval ecdysis. In contrast, selective knockdown of the ETHRB isoform, or suppression of ETHRB-expressing neurons, causes pupal ecdysis deficits that result in lethality. Selective suppression of a small subset of neurons that express ETHRA is sufficient to cause 100% lethality at larval ecdysis. To identify the ETHRA-expressing neuropeptidergic neurons essential for larval ecdysis, we selectively suppressed groups of peptidergic neurons known to express ETHRA. This method failed to identify any single group of peptidergic neurons essential for larval ecdysis, but did identify two groups necessary for normal pupal ecdysis, namely leucokinin- and CCAP-expressing neurons. The latter group has previously been shown to have an essential role in pupal, but not larval, ecdysis, and its function thus clearly varies with developmental stage. Overall, our results demonstrate that the neural circuit governing ecdysis is reconfigured during development and that its dependence on the two ETHR isoforms, and the neurons that express them, shifts between larval and pupal ecdysis.

Disclosures: F. Diao: None. **F. Diao:** None. **J. Shi:** None. **W. Mena:** None. **B. Mark:** None. **D. Park:** None. **P. Taghert:** None. **J. Ewer:** None. **B.H. White:** None.

Poster

471. Invertebrate Motor Circuits

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Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

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Topic: F.04. Neuroethology

Support: MEXT KAKENHI Grant 24120502, 26119501

Title: Population coding of walking locomotion by descending neural activities in the cricket

Authors: *H. OGAWA[1], T. SHUDO[2], M. SOMEYA[2], M. HARUNO[3]
[1]Biol Sci, Fac of Sci., [2]Biosys. Sci. Course, Grad. Sch. Life Sci., Hokkaido Univ., Sapporo, Japan; [3]Ctr. for Information and Neural Network, NICT, Osaka, Japan

Abstract: Descending neural signals regulate motor output for control of speed and direction of locomotion in behaving animals. Information for the locomotion control will be encoded by population activity of large number of descending neurons as the sensory information is also represented by neuronal assembly. However, it has been unknown how and which descending neurons contribute to encode information for the locomotion control, because the cell assembly encoding locomotion in mammals is too huge to be understood about their function and constituent neurons. To address this question, we used the cricket as model system, because small size of insect nervous system allows us to identify whole picture of descending neurons. Crickets perform oriented walking behavior in response to air-current stimuli. Recently, we found that descending signals from cephalic ganglia are required for stimulus angle-dependent control of walking direction and turn angle in the wind-elicited walking behavior (Oe & Ogawa, 2013). To reveal the relationship between population activity of the descending neurons and locomotion, we extracellularly recorded descending spikes from the cricket walking on a spherical treadmill and simultaneously monitor its walking speed and turn angle. Further, we tried to reconstruct the walking speed or turn angle from the ensemble activity of descending neurons using machine learning by sparse linear regression (SLiR) algorithm. Comparing in the decoding performance between voluntary walking and wind-elicited behaviors, the SLiR model constructed from spike activity during voluntary walking predicted the walking velocity in both voluntary and wind-elicited walking with high accuracy. In contrast, the model constructed from wind-elicited walking data set provided high accuracy for prediction of walking velocity in the wind-elicited walking, but did not predict that in the voluntary walking. This fact means that the SLiR model constructed from voluntary walking data set has higher generalization than the model for the wind-elicited walking. That is, the voluntary walking contains a variety of locomotion patterns while the wind-elicited walking is a stereotypical locomotion. The model to predict voluntary walking velocity selected most of the descending units with a light weight, while the model to predict wind-elicited walking velocity heavily weighted fewer units. These results imply that larger number of descending neurons are involved in encoding walking velocity during voluntary walking comparing to wind-elicited walking.

Disclosures: **H. Ogawa:** None. **T. Shudo:** None. **M. Someya:** None. **M. Haruno:** None.

Poster

471. Invertebrate Motor Circuits

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Program#/Poster#: 471.07/VV68

Topic: F.04. Neuroethology

Support: ONR MURI #N000141110725

NSF Graduate Research Fellowship

Title: CPG-driven locomotion of a robotic lobster

Authors: ***D. BLUSTEIN**[1], A. WESTPHAL[2], J. AYERS[1]
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Abstract: Central pattern generators (CPGs) underlying innate animal behavior are common to all motor systems, from sea slugs to humans. In order to understand their operation in natural environments we evaluate their embodied behavior through electronic nervous system simulations in biomimetic robots. Here we present this biorobotic approach by testing hypothetical CPGs underlying walking in a robotic lobster. RoboLobster is an 8-legged underwater walking robot with biomechanics, sensors and a control architecture that mimics that of the American Lobster, Homarus americanus. Each leg of RoboLobster has 6 shape-memory alloy actuators arranged in antagonistic pairs to move three joints. Onboard sensors to detect optical flow, antennal bend (hydrodynamic flow), obstacle contact and heading drive the behavior of the reactively autonomous robot. RoboLobster's electronic nervous system is comprised of mathematically modeled discrete-time map-based neurons and synapses configured to match the known neural circuitry of the lobster. A custom on-board electronics package supports the nervous system simulation configured around command neurons, coordinating neurons and a CPG model that controls omnidirectional walking. Layered exteroceptive reflex networks are activated by sensors that generate a range fractionated labeled-line code and synaptically drive behavioral command neurons. The command neurons modulate pattern generators that rhythmically control the leg's shape memory actuators through motor neurons to mediate adaptations in the vehicle's walking pattern. Coordinating neurons orchestrate the gait between and across body segments. We show that a simple CPG circuit can be modulated to produce adaptive forward, backward, leading, trailing, and diagonal walking and parallel

exteroceptive reflexes. Embodied nervous system simulations give the biologist an amenable platform to test hypotheses and provide the engineer with a tool to help achieve autonomous robotic behavior using biomimetic principles.

Disclosures: D. Blustein: None. **A. Westphal:** None. **J. Ayers:** None.

Poster

471. Invertebrate Motor Circuits

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NIH Grant R01 NS064030

Alfred P. Sloan Foundation Research Fellowship

Title: Degenerate pathways for excitation of the Caenorhabditis elegans pharynx

Authors: *N. TROJANOWSKI, O. PADOVAN-MERHAR, D. M. RAIZEN, C. FANG-YEN
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Degenerate networks, in which structurally distinct elements can perform the same function or yield the same output, are ubiquitous in biology. Degeneracy contributes to the robustness and adaptability of networks in varied environmental and evolutionary contexts. However, how degenerate neural networks regulate behavior *in vivo* is poorly understood, especially at the genetic level. Here, we identify degenerate neural and genetic mechanisms that underlie excitation of the pharynx (feeding organ) in the nematode *C. elegans*. Laser ablation studies have provided key insights into the function of the nearly independent network of 20 neurons that innervates the pharynx, but there are multiple neurons for which ablation causes no obvious phenotype, suggesting they may have degenerate functions. To elucidate the functional connectivity of the pharyngeal network, we developed a technique in which individual pharyngeal neurons in immobilized worms are optogenetically manipulated using selective illumination by a laser beam shaped by a digital micromirror device while behavior is quantified by machine vision. Using this method, we found that individual excitation of each three classes of cholinergic motor neurons, MC, M2, and M4, causes rapid pumping at rates within the range of typical behavior. Using the synaptic wiring diagram and laser ablation, we have demonstrated

that each of these classes of neurons acts directly on pharyngeal muscle to stimulate pumping. Optogenetic inhibition of MC, M2, or M4 decreases pumping rate, confirming a role for these neurons in endogenous feeding regulation. Excitation of the cholinergic I1 interneurons stimulates pumping via MC and M2, and I1 inhibition decreases pumping rate. Previous work indicates that the MC stimulates pumping via a nicotinic receptor. Surprisingly, in worms lacking the nicotinic receptor subunit EAT-2, excitation of MC still increases pumping rate, albeit to a reduced level. Excitation of MC also causes pumping in *eat-18* mutants, which lack pharyngeal nicotinic neurotransmission, but not *unc-17* mutants, which lack all cholinergic transmission, suggesting that MC can stimulate pumping though a non-nicotinic cholinergic mechanism. MC stimulation does not cause an increase in pumping in *eat-18* mutants in the presence of the muscarinic antagonist atropine, nor in double mutants for *eat-18* and the gene encoding the GAR-3 muscarinic receptor. Taken together, these results demonstrate that this robust network is highly degenerate at both the neural and genetic levels, and that multiple classes of neurons can act through different types of receptors to stimulate the same activity pattern *in vivo*.

Disclosures: N. Trojanowski: None. **O. Padovan-Merhar:** None. **D.M. Raizen:** None. **C. Fang-Yen:** None.

Poster

471. Invertebrate Motor Circuits

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Topic: F.04. Neuroethology

Title: Neural mechanisms mediating localization of vibrational signals in the fiddler crab, Uca pugilator

Authors: *A. W. STAFFORD, J. HALL
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Abstract: Many animals use substrate vibration for inter- and intraspecific communication. While the physical properties and behavioral significance of the signals are well documented much less is known about the neural mechanism mediating their analysis, particular with respect to localization of the signal source. To address this issue we have investigated the neural basis of vibrational signaling in the sand fiddler crab, *Uca pugilator*; a species for which vibrational communication is critical for social interactions including mating. Intracellular recording

techniques were used to evaluate the effect of stimulus direction on the response latency of vibration-sensitive neurons (n=36) in the supraesophageal ganglion (brain) of fiddler crabs to stimuli presented at spatially distinct locations around the animal. We focus on response latency as it has been shown to provide, in part, directional information for airborne sounds at the level of the mammalian auditory cortex. Our results identified two populations of neurons; (1) those whose response latency was independent of stimulus location (n=15) and (2) those showing directionally dependent changes in response latency (n=21). We propose that the first population serves to provide an internal temporal reference for signal onset against which the directionally-dependent changes in response latency of the second population can be measured. Thus, these two neural ensembles, like their analogs in animals utilizing airborne communication signals, work in concert to provide directional information about the source of behaviorally relevant vibrational signals.

Disclosures: A.W. Stafford: None. **J. Hall:** None.

Poster

471. Invertebrate Motor Circuits

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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Brains & Fellowship from Georgia state University

Title: The effect of proprioceptive feedback on motor bursts in crayfish

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Abstract: During walking in crayfish, the coxobasal chorodotonal organ (CBCO) provides proprioceptive feedback to the thoracic nervous system. This sensory organ is an elastic strand coding the upward and downward movement of crayfish leg. Extracellular activity of the CBCO sensory nerve, and the depressor (Dep) and levator (Lev) motor nerves of one leg were recorded from an *in vitro* preparation of the thoracic nerve cord. Dep and Lev motor activity excited

corresponding Dep and Lev muscles of a computational neuromechanical model of the crayfish leg (AnimatLab.com), to lower and raise the model leg, respectively. The resulting model leg movements controlled mechanical stimulation of the real CBCO stretch receptor and excited CBCO afferent responses that completed the proprioceptive feedback loop. The feedback loop was closed or opened by coupling or uncoupling motor nerve responses to the corresponding model muscles. Exposure of the preparation to a muscarinic agonist (oxotremorine) induced an active state in which resistance reflexes to leg perturbation reversed to become assistance reflexes, and irregular low-frequency Lev/Dep motor burst pairs occurred when the feedback loop was open. Closing the feedback loop accelerated the motor rhythm three-fold and restructured the motor bursts. The Lev bursts in closed loop began more abruptly, reached higher frequencies and ended earlier than Lev bursts in open loop.The depressor burst that immediately followed each Levator burst was similarly affected. These burst changes produced a more abrupt, faster and shorter leg movement when the feedback was closed. To determine how feedback affects the firing pattern of the individual sensory and motor neurons (MNs) that contribute to the bursting, recorded nerve activity was sorted into spike trains of 18 levator Mns, 12 depressor MNs, the common inhibitor MN, and ~36 CBCO afferents with the analysis program DataView. We found that in closed loop the firing pattern of most MNs changed significantly from the pattern in open loop. For these, the initial firing rate increased more quickly to a higher level but ended earlier during the burst than the same neuron in open loop. For others, there was little difference between the MN's response in closed loop and open loop. The faster rise of the MN's firing in closed loop is likely the result of the assistance reflex that is triggered by the leg movement. The shorter burst duration of some MNs may result from a change in the burst dynamics of the Lev/Dep half-center oscillator.

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Poster

471. Invertebrate Motor Circuits

Location: Halls A-C

Time: Monday, November 17, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 471.11/VV72

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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Title: The effect of presynaptic membrane potential on short-term facilitation

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Abstract: A form of short-term synaptic plasticity of general interest consists of a potentiating effect of holding potential on spike mediated synaptic transmission. We are studying this plasticity in a model system- an *Aplysia* sensory neuron B21 and a postsynaptic follower. Previous experiments demonstrated that effects of holding potential are partially mediated via the activation of a DHP-sensitive calcium current induced by subthreshold depolarization. Activation of this current increases the 'background' [Ca+2]i, which then increases the efficacy of subsequent spike mediated transmission. This phenomenon was initially described in the situation where the presynaptic neuron is firing at a low frequency so that a second form of plasticity, homosynaptic facilitation, is not induced. Since the two forms of plasticity are likely to be co-induced under physiologically relevant conditions, a goal of our more recent work has been to determine how they interact. Previously we demonstrated that depolarizing changes in holding potential impact facilitation in that they increase the rate at which it occurs. Underlying mechanisms have not been described. To determine whether an effect on 'background' [Ca+2]i is involved, we induced facilitation at different membrane potentials and simultaneously monitored calcium levels using imaging techniques. We stimulated B21 using a protocol that enabled us to look at a wide range of instantaneous firing frequencies. Spikes were randomly generated with ISIs between 0.15 and 3.5 seconds. This proved to be an effective protocol for our purposes since facilitation only occurred at the most depolarized holding potential. Increases in [Ca+2]i were greatest at this potential. [Ca+2]i and PSP amplitude were correlated for all conditions, but the relationship was much steeper at the most depolarized potential (i.e., when increases in [Ca+2]i were largest and facilitation occurred). In an attempt to manipulate the [Ca+2]i to determine its impact on facilitation, we performed experiments with nifedipine. Nifedipine decreased the total [Ca+2]i. Further, it impacted synaptic transmission in that we no longer observed significant facilitation when B21 was maximally depolarized. Our data suggest that the DHP sensitive current that plays an important role in regulating the efficacy of synaptic transmission in the absence of facilitation, can also play an important role when facilitation does occur. By impacting the total [Ca+2]i this current can alter the relationship between [Ca+2]i and PSP amplitude making it steep enough to induce significant potentiation of transmission.

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Poster

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Title: The interplay between sensory feedback and intrinsic neural dynamics in a neuromechanical model of motor pattern generation

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Abstract: For animals to robustly produce rhythmic behaviors (walking, swimming, swallowing) in complex, variable environments, central pattern generating circuits must respond dynamically to sensory input. Different dynamical architectures have been proposed to account for rhythmic behaviors including limit cycles, sequences of stable attractors, and stable heteroclinic channels (SHCs). In an SHC, a stable limit cycle passes close to some number of saddle equilibrium points, which can serve as control points for the timing of different components of the oscillatory pattern. We investigated the interplay of central and peripheral factors jointly controlling the feeding apparatus of the marine mollusk *Aplysia californica* in a nominal neuromechanical model. Three pre-motor neural pools control a biomechanical model of the *Aplysia* buccal mass, which in turn provides proprioceptive feedback to the neural pools. The simulated buccal mass feeds on a representation of a seaweed strip (a fixed resisting force), allowing us to measure the feeding efficiency of the model motor patterns. Furthermore, by varying the seaweed force, we can assess how the model responds to changing mechanical loads. We find that for a given value of the seaweed force, a single parameter can be used to switch the neural dynamics between two distinct dynamical regimes, which both produce robust oscillatory behavior. In the heteroclinic dominated regime, proprioceptive feedback from the muscles exposes the "slowing down" associated with fixed points in the neural dynamics, selectively prolonging specific motor phases. In the limit cycle dominated regime, the timing of the pattern is relatively insensitive to proprioceptive feedback. The responsiveness of the system to variable mechanical loads differs significantly in the two regimes. We found that the heteroclinic regime adapts to varying loads more effectively than the limit cycle regime, and this enhanced sensitivity leads to significantly greater net seaweed consumption. Numerical phase response curves computed in the different regimes reveal dramatically different responses to brief neural and mechanical perturbations, suggesting experimentally testable hypotheses for the underlying dynamics of the biological system.

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Poster

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Title: Investigating electrical activity accompanying functional recovery of the pyloric circuit following isolation from neuromodulatory inputs

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Abstract: We use a simple, invertebrate neural circuit to study neural systems' adaptive ability. The pyloric circuit of the stomatogastric ganglion is an example of a central pattern generator that controls muscles needed for food particle sorting in crustaceans. As a central pattern generator, the pyloric circuit controls an oscillatory behavior that must be maintained in the face of a changing environment. A well-studied property of this circuit is its ability to regain a stable activity rhythm after losing it for several days due to isolation from neuromodulatory inputs via decentralization. Previous research supports that this functional rhythm recovery results from readjustment of cells' conductance parameters as both a direct response to loss of neuromodulatory inputs and an indirect response to changes in cells' own electrical activity. While functional recovery is widely observed, specifics of the recovery process are highly variable across preparations. We investigated the circuit's electrical activity patterns following decentralization in order to gain a better understanding of the recovery process. We used continuous extracellular recordings of pyloric activity from Cancer borealis following decentralization in both untreated preparations and preparations treated with chondroitinase ABC, an enzyme known to delay or prevent reemergence of stable rhythm. We looked for differences in post-decentralization electrical activity between treatment groups and for aspects of post-decentralization activity that are predictive of the stable rhythm that is later regained. We developed numerous metrics to quantify how features of pyloric activity such as speed,

regularity, and activity level change over time. Across these many activity metrics, we did not find strong evidence that electrical activity following decentralization differs between treatment groups or is predictive of adaptive ability. The complex interaction of neuromodulator and activity dependent mechanisms that underlie the recovery process may explain why we did not find electrical activity alone to provide information about adaptive ability. Pyloric bouting patterns following decentralization, although highly variable across preparations, have previously been found not to be predictive of recovery. Our results suggest that more gradual changes in post-decentralization activity also are not predictive of the recovery process.

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Poster

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Title: Homeostatic plasticity directed by physical therapy facilitates locomotor recovery after removal of cephalic inputs

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Abstract: Homeostatic plasticity is an important attribute of neurons and their networks, enabling them to recover functional activity after environmental or surgical perturbation. In this study, we determined whether the leech, Hirudo verbana, could recover its ability to crawl after its brain was separated from the crawl central pattern generators it regulates. We observed that coordinated crawl movements returned about 7-10 days after surgery, eventually becoming indistinguishable from motor patterns observed during normal crawling. This recovery was notable because the brain was shown previously to be both necessary and sufficient for leech crawling, especially for the intersegmental phase relationships needed for productive locomotion. Even following complete removal of the brain, we observed recovery of crawling behavior.

Physical therapy involving proprioceptive stimulation decreased the amount of time needed to reach full recovery to as little as two days. During therapy, leeches were squeezed with a molded Sylgard device starting from the anterior regions of the body. These squeezes simulate body deformations that would occur when the circular muscles are active during the elongation phase of normal crawling. However, there were limitations to the therapy regime: first, therapy had to occur in an anterior to posterior direction for an individual to benefit; individuals receiving therapy in the reverse direction recovered no more quickly than those which had not received therapy. Second, while a single therapy session offered some benefits, most individuals required multiple physical therapy sessions to expedite the return of crawling behavior. Lastly, therapy must occur soon after the transection, individuals which received their first therapy sessions at three or more days following the surgery did not recover earlier than their sham counterparts. When the nerve cord in the middle of leech's body was transected, its posterior body showed a loss in the ability to crawl which was not seen in the anterior half. While physical therapy helped the posterior region to regain crawling ability, it never acquired its original coordination with the anterior body. Preliminary data indicate that the dopaminergic system, vital for crawling, may become reorganized to facilitate the posterior-directed wave of coordinated intersegmental activity needed for productive crawling.

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